ECOLOGY AND CONSERVATION OF ALBATROSSES AND PETRELS AT SEA OFF BRAZIL

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DECLARATION

I declare that the work described in this thesis has been conducted independently by myself under he supervision of Professor Robert W. Furness, except where specifically acknowledged, and has not been submitted for any other degree.

This study was carried out according to permits No. 0128931BR, No. 203/2006, No. 02001.005981/2005, No. 023/2006, No. 040/2006 and No. 1282/1, all granted by the Brazilian Environmental Agency (IBAMA), and International Animal Health Certificate No. 0975-06, issued by the Brazilian Government. The Scottish Executive - Rural Affairs Directorate provided the permit POAO 2007/91 to import samples into Scotland.

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Black-browed albatross. Photo: L. Bugoni. Edition: G. Osório.

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SUMMARY

In this study I investigated Procellariiformes (albatrosses, petrels and shearwaters) at sea in the southwestern Atlantic Ocean. Fourteen species and 301 individuals were sampled non-destructively using a cast net method described here. A method is described for ageing Atlantic Yellow-nosed (*Thalassarche chlororhynchos*) and Black-browed (*T. melanophris*) Albatrosses based on bill colour and moult. Procellariiformes appear to have two distinct moulting strategies: petrels and shearwaters have complete annual moult, start to moult during the breeding period, and replace several primaries and tail feathers at one time, whereas albatrosses undergo long moulting cycles, replace less feathers at once, and suspend the wing moult during breeding periods. Primary moult starting at P2 rather than P1 was demonstrated to be a common feature in this taxon, with important implications for studies of stable isotopes, trace elements and pollutants in feathers. Overlap between moulting and breeding is demonstrated to be common with tail and contour feathers, but limited in wing, which suggests that flight constraint in long distance foragers rather than nutritional and energetic limitations is the ultimate factor determining primary moult timing. Based on molecular sexing and linear measurements, sexual size dimorphism was shown to vary according to species, with females in general smaller than males, more pronounced in bill measurements than in other traits, and more conspicuous in Giant Petrels (*Macronectes* spp.) and *Diomedea* albatrosses. Closely related species pairs of *Thallassarche* albatrosses and *Procellaria* petrels had differing levels of sexual dimorphism. The pelagic seabird community sampled comprises birds from different ages and breeding status according to species. Skewed Adult Sex Ratio (ASR) has been proposed as a common pattern in birds, frequently biased towards males and with larger biases in globally threatened species. In albatrosses and petrels, differential mortality of one gender in fisheries is suggested to be caused by sexual size dimorphism giving males a competitive advantage, which allows more access of the larger sex to discards and baits, or to at sea segregation of sexes. These hypotheses were tested by sampling birds at sea and reviewing ASR of birds incidentally captured in fisheries. Skewed ASR is common in albatrosses and petrels from the community attending vessels, but there was no correlation between skewed ASR and conservation status, both in terms of population size or global level of threat, or between ASR and sexual size dimorphism. Thus, sexual dimorphism in size does not explain skewed ASR in the community sampled or in incidental captures in fisheries reported in the review. Differential at sea distributions of males and females appear to explain better the patterns found in the community sampled at sea, as well as skewed ASR in seabird fatalities. Kernel density analysis of satellite tracked Spectacled Petrels (*Procellaria conspicillata*) in 2006 and 2007 demonstrated intense use of waters in the Brazilian Exclusive Economic Zone, from 26 to 31°S, mainly over the continental shelf break and offshore waters. The marine habitats used by Spectacled Petrel and described by bathymetry, SST and productivity are remarkably different from those of the sister

species White-chinned Petrel (*Procellaria aequinoctialis*), which occurs in the area during the winter, but remains over the continental shelf, on Sub-Antarctic and oligotrophic waters. A close association between birds and pelagic longline fishery was demonstrated through comparison of the main kernel areas used by Spectacled Petrels and the pelagic longline fleet. Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) in blood preserved in different ways and simultaneously growing feathers were analysed in Spectacled Petrel. Mean $\delta^{13}C$ and $\delta^{15}N$ values in growing feathers were higher than in blood, suggesting tissue-specific fractionation. Different methods of preserving tissues such as blood may bias stable isotope values. Air drying is proposed as a practical and unbiased method for blood preservation in field situations where freezing is not a practical option, and a mathematical approach is suggested to permit comparison between studies using different preservation methods or tissues. By analysing stable isotopes in blood of all species of Procellariiformes sampled it is demonstrated that availability of discard, mainly the preferred shark liver, which is shared among species with contrasting body masses and feeding techniques, is the ultimate cause of overlapping in trophic levels. Carbon isotopic signature overlapped among the species and was typical of subtropical and offshore region. Early migrant individuals from Antarctica and sub-Antarctic (e.g. Cape Petrel *Daption capense*, White-chinned Petrel, Wilson's Storm-petrel *Oceanites oceanicus*) and species still rearing chicks when sampled (Wandering Albatross *Diomedea exulans*) had clear carbon isotopic signatures from the austral region. All southern latitude species underwent a clear shift in carbon and nitrogen isotope signatures, increasing in trophic level after arriving in the SW Atlantic. Cory's Shearwater (*Calonectris diomedea*), the only species not attending vessels, has low nitrogen values resulting from a diet of flyingfish and squids naturally occurring in the area. While some abundant and widespread petrels and shearwaters, particularly those small in size, benefit from discards from pelagic longline vessels, the balance between benefits and the incidental mortality in hooks is negative for albatrosses and some petrels. Information on bycatch rates of seabirds in the Brazilian domestic pelagic longline fishery from 2001 to 2007 in the Exclusive Economic Zone and adjacent international waters of the southwestern Atlantic Ocean is presented, and bycatch rates for the area are reviewed. Overall, seabird capture rate for the Brazilian pelagic longline fleet was 0.229 birds/1000 hooks, varying from zero to 0.542 birds/1000 according to season. Capture rates were higher between June and November (cold season) and affected mainly the Black-browed Albatross (55% of birds captured), the White-chinned Petrel, the Spectacled Petrel and the Atlantic Yellow-nosed Albatross. Capture rates previously reported in the SW Atlantic varied from 0 to 5.03 birds/1000 hooks, with those based on logbooks or fishermen interviews tending to underestimate capture rates, whereas those based on small numbers of hooks or short time periods tend to greatly overestimate rates in both pelagic and demersal longline fisheries. A range of poorly-known hook-and-line commercial fisheries carried out by the Itaipava fleet, southeastern Brazil, composed by 497 vessels,

was described with seven fisheries defined. Capture rates were higher for the surface longline for Dolphinfish (0.15 birds/1000 hooks and 1.08 turtles/1000 hooks), slow trolling for Bigeye Tuna (0.41 birds/day) and handlining targeting Yellowfin Tuna (0.61 birds/day). Bycatch of 47 seabirds, mainly the endangered Spectacled Petrel, Atlantic Yellow-nosed, and Black-browed Albatrosses, and 45 turtles of four species were recorded. Longline and other hook-and-line fisheries are the major threat for albatrosses and petrels from different origins when foraging in the SW Atlantic Ocean, and require urgent conservation measures.

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Chapter 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

The Brazilian coast stretches 7,000 km, from 4°N to 34°S, adjacent to substantial maritime areas of the Atlantic Ocean (Fig. 1.1). The majority of this area is warm tropical or equatorial, holding important populations of birds which nest in coastal and offshore islands or migrate to the area from North America or Europe. Tropical Pelecaniformes (boobies, tropicbirds and frigatebirds) and Charadriiformes (terns, gulls and noddies) are the main components of the tropical community (Sick 1997). Due to the proximity of the coast, high abundance, wide distribution and logistic facilities, breeding and wintering coastal marine birds have been the focus of considerable research (e.g. Bege & Pauli 1989; Vooren & Chiaradia 1990; Branco 2004; Bugoni & Vooren 2004, 2005; Bugoni *et al.* 2005; Barquete *et al.* 2008).

Tropical waters off Brazil are home to a few species of Procellariiformes - albatrosses, petrels, shearwaters and storm-petrels - which 1) migrate to the area, such as the Manx (*Puffinus puffinus*) and the Cory's (*Calonectris diomedea*) Shearwaters, and winter in this area during austral summer, or 2) birds which cross the area during migration, such as the Great Shearwater (*Puffinus gravis*) on the way to or back from North Atlantic wintering grounds. Only two Procellariiformes breed in Brazil, the Trindade Petrel (*Pterodroma arminjoniana*) on the remote tropical Trindade Island 1,200 km from the coast (Luigi *et al.* 2008), and the Audubon Shearwater (*Puffinus lherminieri*) with a few nests in Fernando de Noronha and Itatiaia Islands (Efe & Musso 2001, Fig. 1.1). The limited populations of these two breeding Procellariiformes and difficulties in assessing breeding grounds have restricted colony-based studies. However, 10 species of albatrosses and 31 species of petrels, shearwaters and storm-petrels occur in Brazilian territory (CBRO 2007). The highest overall richness and diversity of this group occurs in the south due to the influence of the Falkland (Malvinas) Current in southern Brazil, which brings to the area cold and productive waters (Garcia 1998), and makes it suitable for a range of migrants from breeding areas as diverse as Antarctic and Sub Antarctic Islands, Patagonia, Tristan da Cunha and Gough Islands, New Zealand, British Isles, Azores, Madeira and Cape Verde Islands (Neves *et al.* 2006b).

Overall, Brazil is ranked third among the countries with the highest bird richness, with 1801 species documented to occur (CBRO 2007). However, pelagic seabirds in general, and Procellariiformes in particular, are poorly known in Brazil. The incomplete knowledge of seabird status is confirmed by recent records of several species not previously known to occur in Brazil (e.g. Martuscelli *et al.* 1995; Olmos 2000; Petry *et al.* 2000; Bugoni 2006), or others considered rare but subsequently demonstrated to be regular visitors or even abundant in certain areas and seasons (e.g. Carlos *et al.* 2005; Neves *et al.* 2006b; Olmos & Bugoni 2006).

The information on Brazilian pelagic birds obtained to date is mostly based on occasional observations at sea (e.g. Metcalf 1966; Olmos 2000; Olmos & Bugoni 2006) or, more frequently, records of stranded birds on the coast (e.g. Martuscelli *et al.* 1997; Vooren & Fernandes 1989; Petry *et al.* 2000). Some studies have tried to address ecological aspects of albatrosses and petrels, such as diet and pollution, from carcasses stranded on the beach (e.g. Petry & Fonseca 2002; Colabuono & Vooren 2006; Fonseca & Petry 2007; Petry *et al.* 2007, 2008), with inherent limitations of this source of samples for pollution (Wiese & Elmslie 2006) or dietary (Barrett *et al.* 2007) approaches. Not surprisingly, the ecology of Brazilian seabirds has been rarely investigated at sea, and limited to census of birds attending fishing vessels (Olmos 1997; Neves *et al.* 2006b; Olmos & Bugoni 2006), or, more commonly, studies on incidental capture in fisheries with focus on conservation (e.g. Neves & Olmos 1997; Olmos *et al.* 2001) or fisheries (Perez & Wahrlich 2005).

In this study the pelagic community of albatrosses and petrels in southern Brazil and adjacent waters of the southwestern Atlantic Ocean was investigated using a range of traditional field techniques (e.g. morphometric measurements, moult scores) coupled with modern field (e.g. satellite transmitters) and laboratory (e.g. molecular sex determination, stable isotope analysis) techniques. The general aim was to obtain a panorama of ecological relationships of the wintering species in the offshore region through complementary methods, and provide a scientific underpinning for conservation. Studies on seabirds around the world that involve catching birds for banding or attaching devices, or collecting data on diet or blood samples, are strongly biased to the breeding period, especially during the period when birds care for chicks (Shealer 1998; Barrett *et al.* 2007; Peery *et al.* 2008). Biases towards the breeding period are greatest for truly pelagic seabirds such as albatrosses, petrels and penguins, which spend wintering periods far from the coast, and studies carried out away from colonies rely mostly on at sea observations, birds incidentally killed in fisheries or remote sensing devices attached to birds. Thus, this study also aimed to investigate seabirds during the poorly studied wintering period and to contribute a better understanding of seabird ecology in a global scale.

Procellariiformes in general and albatrosses in particular have suffered severe population declines in historical and contemporary times (BirdLife International 2004), particularly for albatrosses of which 19 out of 21 species are threatened by extinction (BirdLife International 2008). Historical declines are mainly attributed to harvesting in colonies and the introduction of exotic animals on islands, such as cats, rats, mice, and goats (see review in Gales 1997). Whereas these threats are still the major problem for some species and islands, particularly small-sized species, such as *Pterodroma* and storm-petrels (e.g. Cuthbert & Hilton 2004), incidental mortality in fisheries is recognized as the main conservation problem (Gales 1997; BirdLife International 2008). The responsibility

of fisheries in the decline of several Procellariiformes is well documented through decades of monitoring of albatross and petrels populations on the breeding grounds (e.g. Gales 1997; Croxall & Gales 1997; Delord *et al.* 2008), several studies documenting and quantifying the incidental mortality in a range of fisheries (see reviews in Cooper *et al.* 1998; Bugoni *et al.* 2008d, Chapter 9 & 10) and models demonstrating or predicting the impacts of fishery bycatch on demography parameters and population trends (Inchausti & Weimerskirch 2001; Cuthbert *et al.* 2003b).

The innovative approach of studying birds at sea allows us to address distinct subjects not investigated at colonies, but was not without difficulties. The first step was to develop or adapt methods making them practical and safe at sea when sampling onboard of fishing vessels.

The primary difficulty was to obtain samples of a range of seabird species non-destructively, i.e. to capture and handle them safely, which was done using a cast net and methods described in **Chapter 2**.

Customary methods applied ashore, such as linear measurements, moult scores, banding and blood samples taken with syringe and needle were, in general, feasible at sea, except body mass measurement or under rough seas, where trapping and sampling was difficult and dangerous for birds and the researcher. To investigate the age composition of birds in the community I collected information on moult and plumage changes according to age described previously. However, for one albatross species I described a potential method for ageing based on bill colour and moult and provided further evidence of population differences in another species (**Chapter 3**). I was also interested in ageing birds for assigning groups of samples according to age classes of birds for stable isotope analysis.

As most Brazilian fishing vessels are quite small (15-25 m long) and with restricted facilities onboard, an additional problem was preservation of blood for molecular or isotope analysis, as a power supply for freezing was intermittent or not available. Thus, in the third methodological section (**Chapter 4)** I compared stable isotope signatures of carbon and nitrogen using different preservatives and methods, and their suitability for studies in remote field places.

The following three chapters deal with general biology based on information collected onboard or samples subsequently analysed in laboratory. The first one (**Chapter 5**) compares moult patterns of albatrosses and petrels when in active moult during wintering and breeding periods and how life-strategies differ according to taxa. In **Chapter 6**, morphometric measurements and age data were used to extend knowledge

of the age classes of birds present off Brazil, and investigate sexual size dimorphism of birds sexed by molecular methods. Carbon and nitrogen stable isotopes in blood of 14 albatrosses and petrels were determined and trophic structure analysis was carried out. Resource partitioning and segregation between species, ages and sexes was further addressed (**Chapter 7**).

Among birds, albatrosses (Diomedeidae) are the most endangered taxon, mainly due to incidental capture in fisheries. The final four chapters have a conservation perspective, from capture rates in fisheries to the use of satellite telemetry, and biased sex-ratio in birds captured in fisheries.

Thus, in **Chapter 8** I described the marine habitats of Spectacled Petrels (*Procellaria conspicillata*) based on satellite transmitters attached on birds at sea, how bird distribution is related to oceanographic variables, such as sea surface temperature, productivity and bathymetry, and species segregation with the closely related White-chinned Petrel (*P. aequinoctialis*). Fine-scale fishing effort for the pelagic longline fishery was obtained from logbooks and fishing effort compared with petrel locations.

Capture rates of seabirds in Brazil are presented in **Chapter 9**, making use of the most complete dataset available to date for the area, from 2000 to 2007. Capture rates per species are presented and compared according to season and bird abundance. I also review all capture rates reported for longline fisheries in the southwestern Atlantic Ocean and discuss differences according to the fisheries, their representativeness as descriptors of captures in the region, and gaps that remain for further studies and conservation.

Apart from the relatively well known pelagic and bottom longline fisheries, small scale hook-and-line fisheries are also an additional cause for concern. The bycatch in small scale fisheries has been neglected worldwide, but their potential effects on seabirds and other groups, such as sea turtles is potentially high. In **Chapter 10** I described a range of hook-and-line techniques used by a fishing fleet of about 500 vessels along the Brazilian coast to target pelagic and demersal fish species. I assessed their differential threats to birds and turtles through preliminary capture rates and provide a discussion on conservation and potential impacts of such activities.

Finally, in **Chapter 11** I provided sex ratios of birds captured using cast nets and sexed through molecular methods, and reviewed reported sex ratio for a range of albatrosses and petrels in different fisheries. Results were discussed with respect to postulated hypotheses found in literature, i.e. sexual size dimorphism as determinant of accessibility to discards and baits, and differential at-sea distribution according to sex.

All chapters mentioned above are independent of each other, as prepared for publication, and most of them are currently under review, in press or published. Unavoidably there is therefore considerable repetition across chapters, particularly in introduction and methods sections. To avoid further repetition I provide a single list of references at the end of the thesis, and the general discussion in **Chapter 12** addresses general topics of the thesis, potential sample biases and discuss briefly how we improved knowledge of marine birds off Brazil after this study.

Appendix 1 addresses a subject closely related to this thesis and with samples collected during its development, but as it was not primarily written by me, it was not included in the main body of the thesis.

During the development of the thesis I had the opportunity to analyse data from the effects of a hurricane on petrels. It is related to the topics investigated in the thesis, but because sampling was opportunistic and differs considerably from the rest of the thesis, I present it as in **Appendix 2** rather than a main chapter.

In **Appendix 3** I discuss other studies carried out during the thesis, but not included here as substantial chapters, particularly studies on breeding Trindade Petrels (*Pterodroma arminjoniana*) on Trindade Island, Brazil.

Figure 1.1. Map of South America and the southwestern Atlantic Ocean, with localities mentioned in the text.

Chapter 2

AN EFFECTIVE METHOD FOR TRAPPING SCAVENGING SEABIRDS AT SEA

Bugoni L, Neves TS, Peppes FV, Furness RW (2008b) An effective method for trapping scavenging seabirds at sea. ***Journal of Field Ornithology*** 79(3): 308–313.

ABSTRACT

Most studies of seabirds that involve trapping and marking birds are carried out at breeding colonies. This bias towards the breeding period and colony-based research is partially caused by difficulties in capturing birds at sea. From 2005-2007, we used a cast net thrown by hand from a fishing boat to capture albatrosses and petrels at sea in the southwestern Atlantic Ocean. About 500 birds of 13 species were captured, ranging in size from the 30-g Wilson's Storm-petrel (*Oceanites oceanicus*) to the 10-kg Wandering Albatross (*Diomedea exulans*). Cast nets are potentially useful for capturing any seabird that can be attracted close to fishing vessels by bait, such as sardines, squid, offal, or shark liver, thrown into the water. Our method was most effective for capturing bold species, such as Cape (*Daption capense*), Spectacled (*Procellaria conspicillata*), and White-chinned (*Procellaria aequinoctialis*) Petrels and Great Shearwaters (*Puffinus gravis*), but was not effective for capturing shy species, such as Cory's (*Calonectris diomedea*), Cape Verde (*Calonectris edwardsii*) and Manx (*Puffinus puffinus*) Shearwaters, species that rarely sit on the water, such as Wilson's Storm-petrels, Thin-billed (*Pachyptila belcheri*) and Antarctic (*Pachyptila desolata*) Prions and Atlantic Petrels (*Pterodroma incerta*), and species with excellent diving abilities, such as Sooty Shearwaters (*Puffinus griseus*). For many species of seabirds, cast nets would likely be more efficient for capturing large numbers of birds than other methods, such as hoop nets.

INTRODUCTION

Most studies that involve catching seabirds for banding, attaching devices, or collecting data on diet have been conduced during the breeding season, especially the period when birds care for chicks (Shealer 1998; Barrett *et al.* 2007). This is particularly true for pelagic seabirds, such as albatrosses, petrels, and penguins that spend non-breeding periods far from the coast. Recently, investigators have used remote sensing devices to study birds during the non-breeding period, with data recovered through satellites, or data stored in loggers during the entire non-breeding period and downloaded when birds return to breeding colonies (Wilson *et al.* 2002). However, geolocators provide only coarse-scale information, accurate to several kilometres (169 - 186 km; Phillips *et al.* 2004a), and the ability to capture birds and attach satellite-transmitters at sea during the non-breeding period would provide considerable advantages in terms of obtaining fine-scale information on movements of individuals and interaction with fisheries. Thus, the development of techniques for non-destructive sampling of seabirds at sea would help improve our knowledge of seabird ecology during the non-breeding period.

Gill *et al.* (1970) used a hand-thrown net (hoop net) to capture shearwaters and storm-petrels at sea, and Gibson & Sefton (1959) used a similar method to capture albatrosses and petrels resting on water. More recently, Suryan *et al.* (2007) used hoop nets to

capture Black-footed (*Phoebastria nigripes*), Laysan (*Phoebastria immutabilis*), and Short-tailed (*Phoebastria albatrus*) Albatrosses, with a dip net used to haul birds onboard (K.N. Fischer, *in litt.*). However, hoop nets typically permit capture of few birds (or just one) and may require up to three people to operate (Gibson & Sefton 1959; Gill *et al.* 1970; K.N Fischer, *in litt.*). Here we describe an effective and safe method for capturing albatrosses and petrels from vessels at sea, and discuss their potential application for capturing other seabird species.

METHODS

We captured birds in the southwestern Atlantic Ocean (25 to 35ºS) over the continental shelf and offshore waters of southern Brazil. The region is under the influence of the Subtropical Convergence, formed by the meeting of the warm tropical Brazil Current flowing southward and the cold Falkland (Malvinas) Current flowing northward (Garcia 1998). The mixing waters of two distinct currents and continental waters form an oceanographic mosaic with steep gradients of temperatures and productivity, and support a large fishing fleet that attracts scavenging seabirds to feed on discards and offal. Ten species of albatrosses and 31 species of petrels occur in these waters (CBRO 2007), mainly in the southern area. Trapping was carried out from commercial fishing vessels during their normal operations.

Description of the net and trapping methods

The cast net we used to capture albatrosses and petrels is the typical net used by fishermen to capture fish and shrimp in shallow waters (FAO 1982; Perrow *et al.* 1996). It consists of a circular net with weights attached to the perimeter, and a rope attached to the center of the net in one end, and held at the other end by the fishermen (Figs. 2.1 and 2.2). The net is thrown on to the water's surface, swirling it so the net opens and captures the target by falling on and entangling it (Perrow *et al.* 1996). There are basically two designs: the simple one described above that captures fish as the net collapses and another, more sophisticated, with internal purse-lines connecting to the perimeter of the net with the rope attached to the midpoint that draws the net together during hauling. This latter model is equipped with a metal ring at the center from where the purse lines slide. The connection between the rope and the purse lines is usually by a swivel. Because fish or birds are trapped in the pockets formed during hauling, this design is generally more effective. Both cast net designs were used, and they were 3.5 - 5 m in diameter with 25 – 35 mm mesh and weighed 2.5 - 4 kg.

RESULTS

Cast nets were initially used to capture seabirds based on suggestions from fishermen, and, in 2005, were used to capture 183 Spectacled Petrels (*Procellaria conspicillata*) for a genetic study. On 58 days during the periods from February to June 2006 and August-

September 2007, we used cast nets to capture albatrosses and petrels attracted to a fishing vessel using sardines, squid, or offal. Shark liver was found to be effective because it floats and is apparently preferred by birds. Lines were attached to bait to keep it close to the vessel. When attempting to capture birds, one person attracted the birds by casting bait or tethering the liver/offal and another person handled the net.

Most captured birds were sitting on the surface of the water, but a few were captured in flight. Capture distance varied with wind conditions and the skill of the person handling the net, but most captured birds were 4 to 8 m from the vessel (with the person throwing the net positioned 2 m above the water). To capture birds, the vessel must be drifting or moving as slowly as possible.

We captured 296 birds representing 13 species from two fishing vessels (Table 2.1). Vessels were 24 and 26 m long and using a variety of hook and line fishing methods or pelagic longline (Bugoni *et al.* 2008a, c). Additional birds were captured (~80), but were released because they were either non-target species or more birds were captured than could be processed. Species captured were as small as a 30-g Wilson's Storm-petrel (*Oceanites oceanicus*), and as large as a ~10-kg Wandering Albatross (*Diomedea exulans*). Although we once captured 23 birds with one net, including one Southern Giant Petrel (*Macronectes giganteus*), one Black-browed Albatross (*Thalassarche melanophris*), and 21 Cape Petrels (*Daption capense*), and, on another occasion, captured two Wandering Albatrosses (Fig. 2.3), we usually tried not to capture several birds simultaneously to avoid unnecessary stress to the birds and because of our time-consuming sampling protocol. However, if needed, we probably could capture over 100 birds in a day and over 40 birds in a single throw, particularly tame and abundant species like Cape and White-chinned (*Procellaria aequinoctialis*) Petrels. Frequently, we were able to capture particular species or individuals in a flock when birds came close to the vessel. Thus, we were often successful at targeting particular individuals to increase sample size for certain species or to capture previously banded birds. However, this was not always possible, particularly for species like Royal Albatrosses (*Diomedea epomophora* and *D. sanfordi*) and Northern Giant Petrels (*Macronectes halli*).

Our method was most effective for bold species such as Cape, Spectacled, and White-chinned Petrels and Great Shearwaters (*Puffinus gravis*), but was not effective for capturing shy species, such as Cory's (*Calonectris diomedea*), Cape Verde (*Calonectris edwardsii*), and Manx (*Puffinus puffinus*) Shearwaters, species that rarely sit on the water, such as Wilson's Storm-petrels, Thin-billed Prions (*Pachyptila belcheri*), Antarctic Prions (*Pachyptila desolata*), and Atlantic Petrels (*Pterodroma incerta*), or species with excellent diving abilities, such as Sooty Shearwaters (*Puffinus griseus*). We tested both

cast-net designs, and found that the net with seine lines increased capture efficiency, with more birds captured simultaneously and birds less likely to escape.

After capture, birds were kept in boxes lined with newspaper or cardboard to keep them dry. For each captured bird, we took standard measurements, checked for moult, collected blood and contour feathers, and banded them with metal bands. We also took pictures of albatrosses and Giant Petrels for ageing, and some Spectacled Petrels were fitted with satellite transmitters. No birds were captured during periods of high wind (Beaufort scale four or over) or rain to avoid releasing wet birds and to minimize potential injuries. Large seabirds more dependent on wind for take off, such as Wandering and Tristan (*Diomedea dabbenena*) Albatrosses, need to be captured and handled carefully because wetting their plumage can make it difficult for them to get airborne. If their plumage does get wet, they should be kept until dry and then released.

Spectacled Petrels and Great Shearwaters that we banded were subsequently observed or captured a few days after release, and one banded Spectacled Petrel was recaptured four months later. In addition, three adult Wandering Albatrosses that we captured in August were breeding on the South Georgia Islands and all returned to their colonies and successfully raised young (A. Wood and R. Phillips, *in litt.*).

The method of capturing seabirds that we describe caused no injuries to any birds captured. However, on two occasions, the rope that attaches to the wrist came loose as nets were thrown, and nets were lost at sea. Lost nets could potentially entangle and drown birds. However, loss of nets can be avoided by carefully checking the attachment, by tying two knots, or preferably by casting the net with a long safety line attached to the vessel.

DISCUSSION

Using cast nets, we were able to successfully capture nearly 500 birds representing 13 species that ranged in size from Wilson's Storm-petrels to Wandering Albatrosses. We believe that the method we describe would potentially be useful for trapping any seabird that can be attracted close to vessel. Casting the net does require practice to maximize the area sampled (Perrow *et al.* 1996). However, anyone with a few days of practice should be able to use the net successfully.

The cost of cast nets varies with location, model, and net size, but is usually found in fishing gear shops priced between $50 - $130 (US). The locally named '*tarrafa*' net is used in Brazil all along the coast for capturing shrimp, mullets, silversides, and sardines and in freshwater areas for capturing several species of fish. The use of cast nets for capturing birds for research may be authorized by the Brazilian Environmental Agency

(IBAMA-CEMAVE) on a project-specific basis, but further regulations on their use for fishing are applied according to areas or species. Investigators using this method elsewhere must be aware of the legal issues concerning their use in particular countries.

Our results indicate that cast nets represent a safe, inexpensive and effective method for capturing albatrosses and petrels, and we are confident that they could be used to capture other seabirds, such as gulls (*Larus* spp.), Northern Fulmars (*Fulmarus glacialis*), Kittiwakes (*Rissa tridactyla* and *R. brevirostris*), Skuas and Jaegers (*Stercorarius* spp.), Northern Gannets (*Morus bassanus*), and any other species attracted by fishing discards and offal. Using cast nets to capture seabirds at sea should enhance studies of the behavior and ecology of seabirds during the wintering period when they are away from breeding colonies.

Table 2.1. The number and species of albatrosses and petrels captured using cast nets in southern Brazil in 2006 and 2007.

Species	Number captured
Wandering Albatross (*Diomedea exulans*)	9
Tristan Albatross (*Diomedea dabbenena*)	4
Black-browed Albatross (*Thalassarche melanophris*)	33
Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*)	33
Southern Giant Petrel (*Macronectes giganteus*)	11
Northern Giant Petrel (*Macronectes halli*)	1
Spectacled Petrel (*Procellaria conspicillata*)	64
White-chinned Petrel (*Procellaria aequinoctialis*)	30
Cape Petrel (*Daption capense*)	30
Southern Fulmar (*Fulmarus glacialoides*)	10
Wilson's Storm-petrel (*Oceanites oceanicus*)	1
Great Shearwater (*Puffinus gravis*)	68
Sooty Shearwater (*Puffinus griseus*)	2

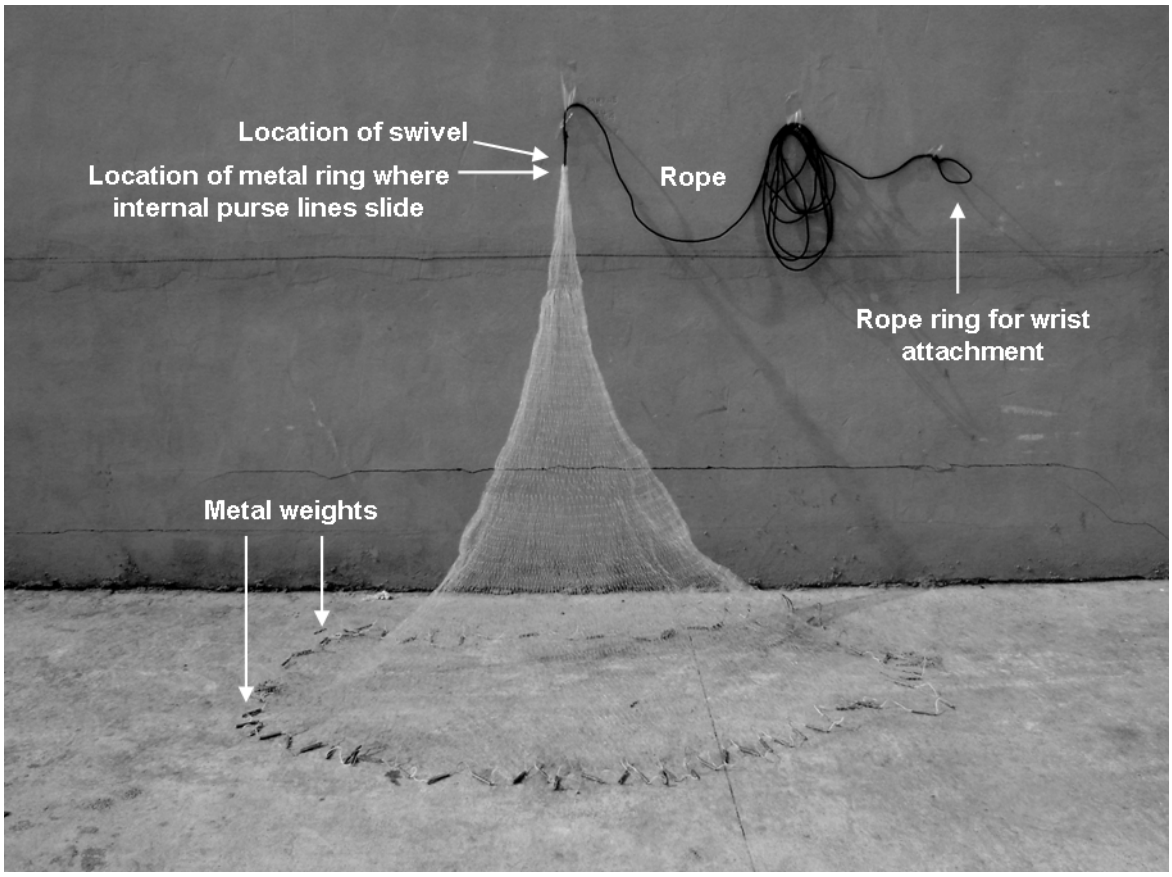

Figure 2.1. Illustration of a cast net used for trapping seabirds at sea in Brazil in 2006 and 2007. The cast net displayed has the simplest design, but locations of the metal ring where seine lines slide and location of the swivel are indicated. Photo by F.V. Peppes.

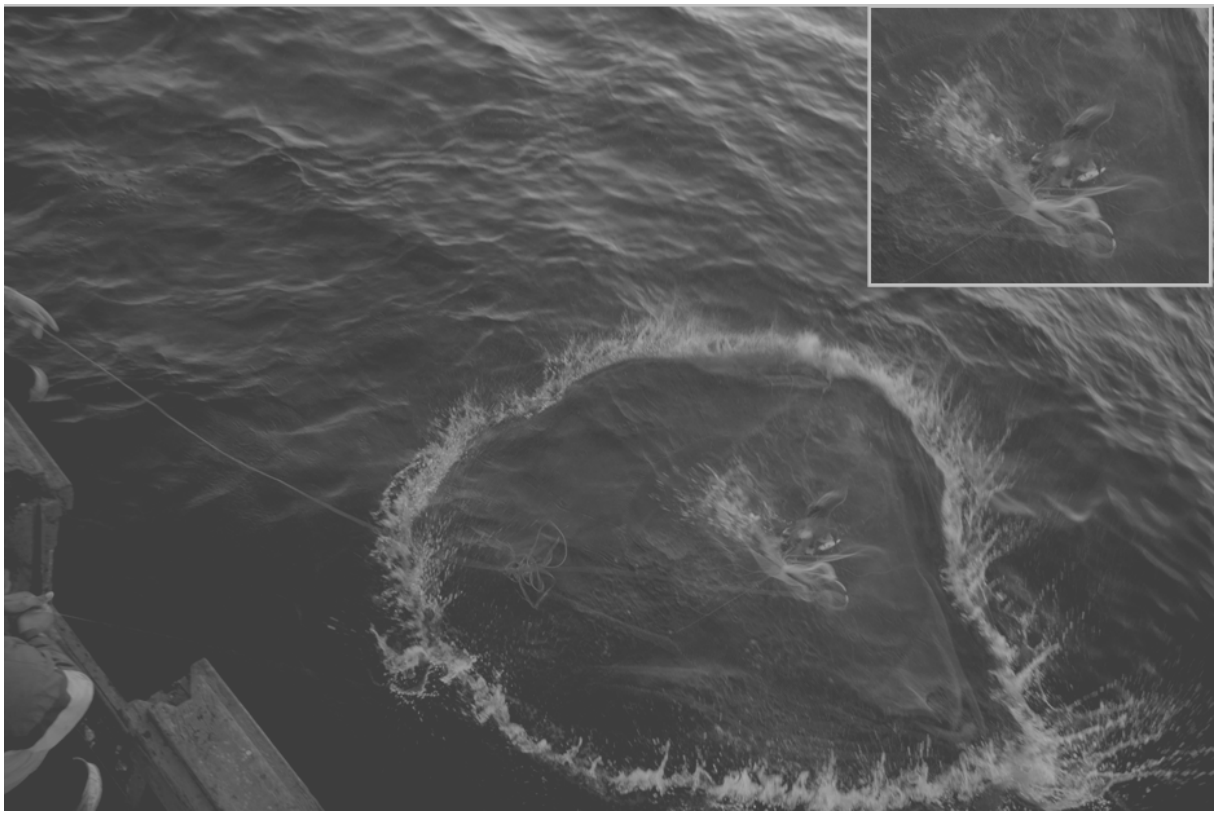

Figure 2.2. Cast net at the moment when it touches the sea surface for the capture of a Great Shearwater (*Puffinus gravis*) in southern Brazil. Note the sardine used for attracting the bird close to the vessel (inset). Photo by L. Bugoni.

Figure 2.3. Two Wandering Albatrosses (*Diomedea exulans*) captured at sea with a single throw of a cast net and being hauled onto the vessel. Nearby are White-chinned, Spectacled, and Cape Petrels and another *Diomedea* Albatross. Photo by M. Oliveira (NEMA).

Chapter 3

BILL COLOUR AND MOULT AS A POTENTIAL TOOL FOR AGEING IMMATURE ATLANTIC YELLOW-NOSED (*THALASSARCHE CHLORORHYNCHOS*) AND BLACK-BROWED (*T. MELANOPHRIS*) ALBATROSSES IN WINTERING GROUNDS

Bugoni L, Furness RW. Bill colour and moult as a potential tool for ageing immature Atlantic Yellow-nosed (*Thalassarche chlororhynchos*) and Black-browed (*T. melanophris*) Albatrosses in wintering grounds. **Under review**

ABSTRACT

Based on birds sampled at sea off the Brazilian coast, we provide a potential method for ageing Atlantic Yellow-nosed Albatrosses (*Thalassarche chlororhynchos*) using bill colour and moult of wing, tail and contour feathers. First year birds have a black bill and show no moult. One year olds have some yellow at the culminicorm, and show tail and contour feather moult but not primary moult. Two year olds show more yellow on the bill progressing through to full orange culminicorn and reddish nail at Age 3, but three year olds differ from adults by having only a pale vertical bar at the base of ramicorn and flight feather moult during the breeding period. Adults have a more pronounced vertical bar at the base of the ramicorn, and do not moult flight feathers while breeding. Comparison with previous descriptions for ageing Black-browed Albatross (*T. melanophris*) is also provided, showing that birds off Brazil (originating mainly from the Falkland (Malvinas) Islands) differ considerably from South Georgia Island birds. First year birds wintering off southern Brazil show paler bills, with colour changing quickly after fledging. However, Black-browed Albatrosses 2-years old with fully black bills as those described from South Georgia Islands were also sampled, suggesting a variety of origins of Black-browed Albatrosses wintering in the area and inter-colony differences in bill colour maturation.

INTRODUCTION

Albatrosses are known to have slow plumage maturation, which last up to 20 years in the large Wandering Albatross (*Diomedea exulans*) (Prince *et al.* 1997b), and even longer in females of the Tristan Albatross (*D. dabbenena*), which are known as breeding birds sometimes still to have chocolate-brown plumage similar to chicks they are rearing (Cuthbert *et al.* 2003a). In the group of small albatrosses, or mollymawks, maturation occurs by increasing the amount of white in the surface of the underwing, as well as changes in bill colour, usually from dark to pale or colourful bill plates. Plumage maturation in mollymawks lasts up to six years, can make age assignment difficult, and is not fully described in most species. For example the Black-browed Albatross (*Thalassarche melanophris*) changes bill colour with age, which combined with examination of the state of flight feather moult allows age determination up to six years-old, as the pattern and timing of flight feather moult change with age up to breeding age (Prince & Rodwell 1994). In a similar way, bill colour maturation and development of moult patterns were described for Gray-headed Albatrosses, based on ringed birds of known age visiting the colonies which change from having a wholly dark bill to orange at the culminicorn, ramicorn and ungues (Prince & Rodwell 1994). Ageing of Atlantic Yellow-nosed Albatrosses (*T. chlororhynchos*) has not previously been described, but because its bill colour is similar to that of the Gray-headed Albatross and also changes from wholly black to orange according to age, we used this characteristic coupled with moult data to describe the characteristics of birds of different ages, from fledging plumage up to adult

plumage, from study of birds captured at sea. This description is expected to facilitate studies describing the age composition of birds through at sea observations, and to assist with ageing of birds incidentally captured in fisheries and of birds washed ashore. It could also be useful for ageing the sister species, the Indian Yellow-nosed Albatross (*T. carteri*), which migrates to Australian and New Zealand waters (Onley & Scofield 2007). For ageing Black-browed Albatrosses we tried to use the description provided by Prince & Rodwell (1994), which was based on known-aged birds captured on breeding grounds, but because we found divergences between our data and the description they provided, we report here a detailed description of bill characteristics and moult we have found and discuss potential reasons for differences between their observations and ours.

METHODS

Study area and seabird capture

Birds were captured over the continental shelf and offshore waters in southern Brazil in the region under the influence of the Subtropical Convergence, formed by the meeting of the warm tropical Brazilian Current flowing southward and the cold Falkland (Malvinas) Current flowing northward (Garcia 1998). Work was carried out onboard fishing vessels targeting Tuna (*Thunnus* spp.), Sharks (mainly Blue Shark *Prionace glauca*) and Swordfish (*Xiphias gladius*) using a range of hook-and-line and pelagic longline fishing methods as described by Bugoni *et al.* (2008c). Birds were captured by cast net after attracting them close to the vessel with baits and shark liver (Bugoni *et al.* 2008b). Sixty-six albatrosses (33 of each species) were captured during six cruises and 58 trapping days from February to June 2006 and July to September 2007, from 25 to 35ºS. A few birds (about ten) incidentally captured on long-line hooks, dead or alive, were also sampled onboard.

Plumage moult and ageing

Moult scores for each of the 10 primary feathers of the right wing (from the inner P1 to the outer P10) and all tail feathers were recorded according to age and stage of development (Ginn & Melville 1983). For every feather a score from '0' to '5' was given, as follows: 0 - old feather remaining; 1 – old feather missing or new feather in pin; 2 – feather emerging from the sheath up to 1/3 grown; 3 – new feather between 1/3 and 2/3 grown; 4 - new feather >2/3 and with remains of waxy sheath at its base; 5 – new feather fully developed with no waxy sheaths remaining at the base. The vestigial 11^{th} primary feather present in Procellariiformes (Marchant & Higgins 1990) was not scored. Distinguishing old from fully grown new feathers is sometimes difficult, but is assisted by the colour and brightness of the feather, which is generally paler and has a ragged tip in old feathers. Moult of contour feathers on head, dorsum and ventral parts was recorded as occurring or not, as was the presence or absence of a brood patch. It was assumed that wing moult is symmetrical, which is generally the case in albatrosses (Furness 1988;

Prince *et al.* 1993) and so moult scores were recorded only for the right wing. Pictures of the bill in lateral and upper view were taken for every bird. Age classes of birds are named as in Prince & Rodwell (1994), which were based on ringed birds of known age.

RESULTS

Ageing and moult of Atlantic Yellow-nosed Albatrosses

The stages from fledging to adult plumage at five-years old, when they recruit (Cuthbert *et al.* 2003b), based on moult timing and bill colour can be described as follows:

First year – From fledging until May of the next year. Bill wholly black, except for a slight pale dirty patch at the tip of upper unguis and at the base of the culminicorn; vertical bar at the base of ramicorn (lower mandible) hard to notice and very pale (Fig. 3.1). No moult occurs.

Age 1 – Pale yellow patch at the ungues tips and at the base of culminicorn (sometimes striped black and yellow) visible at distance and spreading toward the nail. Extension of yellow is variable, but still with black usually halfway along the bill towards the top of the unguis. No primary or secondary feather moult occurs, but some tail and contour feather moult may be present (n = 7 birds, with birds fledged in the previous year, i.e. Age 1 + 1 month sampled in May, included in this category).

Age 2 – Yellow spread along the whole culminicorn, except at the top of the maxillary unguis, but yellow still 'dirty'. First primary moult takes place during austral summer, tail and contour moult take place again. Primary moult occurs in two descendant waves according to the age and size of feathers (n = 7 birds).

Age 3 (pre-breeding) – Bill colour much as adult. Yellow parts of the culminicorn turn orange as in adults, except for vertical bar at the ramicorn, which could remain pale or yellowish. Moult differs from that of adults by occurring during late summer and finishing during autumn, which is when breeders only start to moult (n = 2, one bird in February with bill as adult and moulting primaries, tail and contour feathers; another in June with culminicorn as adult, but vertical bar still pale, moulting primary, tail and contour feathers).

Age 4+ (Adult) – Culminicorn and vertical bar at the base of ramicorn fully orange, borders well delimited and without sign of black. Maxillary unguis is red with pale tip, mandibular unguis pale. No moult during the austral summer when they breed, but moult could start in autumn at the end of breeding period (~April/May). Some moult of contour feathers could take place during breeding (n = 16 breeding birds sampled from February to May and one post-breeding adult sampled in August).

Ageing and moult of Black-browed Albatrosses

Twenty-seven first year Black-browed Albatrosses with no primary, tail or contour moult were captured in April, May and August. Bill colour in May when they were recently fledged was dark grey (horny) with blackish ungues, but in August they already had a very pale bill with black only at the base of culminicorn and ungues, suggesting a rapid change in bill colour (Fig. 3.2). During the same period, Age 1 birds were captured and differed from first year by having moulting rectrices and contour feathers in May, but only contour feathers in August. Primary feathers were all of the same age and birds do not appear to moult P8-P10 and some inner primaries during the second winter as suggested previously (Prince *et al.* 1993; Tickell 2000), but show moult similar to the pattern described for Atlantic Yellow-nosed Albatrosses above. Age 1 birds have bill pale beige with some grey patches, ungues still black, but with pale tips. Both first year (from August onwards) and Age 1 birds are not predominantly dark billed ('bronzy brown') as postulated by Prince & Rodwell (1994). However, one bird captured in April with new rectrices and moulting contour feathers (thus Age 1) had a black bill, even darker than first year birds trapped in May/early June (Fig. 3.2). This bill colour pattern is out of the expected range of birds with this age found off Brazilian coast and could suggest that this individual was from South Georgia whose pattern is similar (Prince & Rodwell 1994) or elsewhere. Two other birds Aged 1 based on moult captured in the same week with typical bill colour, and the small size for a male of this unusually coloured bird confirm that this was an atypical bird.

Two adults captured in late July had the typical reddish unguis and no grey on the bill (Fig. 3.2). All rectrices had grown recently and the primaries had blocks of old and new feathers, with active moulting only recorded in contour feathers. These birds were probably finishing moult in preparation for breeding which starts in September. Only six birds of intermediate ages (immatures) were captured.

DISCUSSION

Bill colour was useful for ageing Atlantic yellow-nosed and Black-browed Albatrosses, but due to individual variations it should be used carefully, and in association with moult data, which provide a clear indication of status as pre-breeding birds. It also could vary according to light and the perception of different observers. The ageing guide based on bill colour and moult proposed here and by Prince & Rodwell (1994) is valid as an approximation for age determination although still remains to be confirmed in birds of known-age and in populations of different origins. This study at sea benefited from birds being in active moult, which is not usually observed in colonies; somewhat counterintuitively, age determination is easier for birds at sea than at colonies for this reason. We could determine breeding status and age based on moult, and detecting new

and old feathers, rather than ageing feathers from first to fifth generations, which is not always possible. By trapping birds at sea in wintering grounds we probably sampled Black-browed Albatrosses from different populations (Falkland (Malvinas) and South Georgia breeding colonies). One out of 33 Black-browed Albatrosses being originated from South Georgia is in accordance with the 6% of ring recoveries of young birds from this island occurring in the SW Atlantic Ocean (Prince *et al.* 1997a). Because bill colour consistently matched moult patterns for each age category assigned in both species the method presented here is potentially useful for ageing determination of age composition of birds incidentally captured in fisheries and beach stranded birds. It is also potentially applicable to the Indian Yellow-nosed Albatross wintering in Australian waters, but further validation of that is required.

It is remarkable that 59% of Atlantic Yellow-nosed Albatrosses captured during the breeding season in Brazilian waters were adults, and that 48% had no primary or rectrix moult (i.e. were breeding adults), which means that breeding birds make long foraging trips to the SW Atlantic Ocean during breeding. In this area, breeding birds meet a range of hook-and-line and longline fishing vessels which provide discards, but also where they are at risk of being drowned on hooks (Bugoni *et al.* 2008a). The population is in decline, mainly due to incidental capture in fisheries (Cuthbert *et al.* 2003a).

First year Black-browed Albatrosses composed 2/3 of our at sea captures, with minor numbers of immatures and juveniles. These proportions in captures reflect the abundances of different ages observed at sea (Neves *et al.* 2006b), as well as the age composition of birds captured on pelagic longlines (Neves & Olmos 1997) and stranded on the southern Brazilian coast (Colabuono & Vooren 2006). Many are known to originate from the Falkland (Malvinas) Island, as demonstrated by records of chest painted fledglings (Sullivan *et al.* 2003). Thus, the odd Age 1 Black-browed Albatross we caught with a distinct darker bill could possibly originates from South Georgia, where immatures have darker bills (Prince & Rodwell 1994) or from elsewhere. This implies differences in bill colour between colonies and is in line with significant genetic differences between Falkland (Malvinas) and South Georgia birds (Burg & Croxall 2001). Furthermore, some adults from South Georgia are known to winter in the SW Atlantic rather than the more common migration to the Benguela system off South Africa (Phillips *et al.* 2005b). The suggestion that birds with distinct origins have different bill colour maturation requires further studies regarding the applicability of the description of age classes across Black-browed Albatross populations.

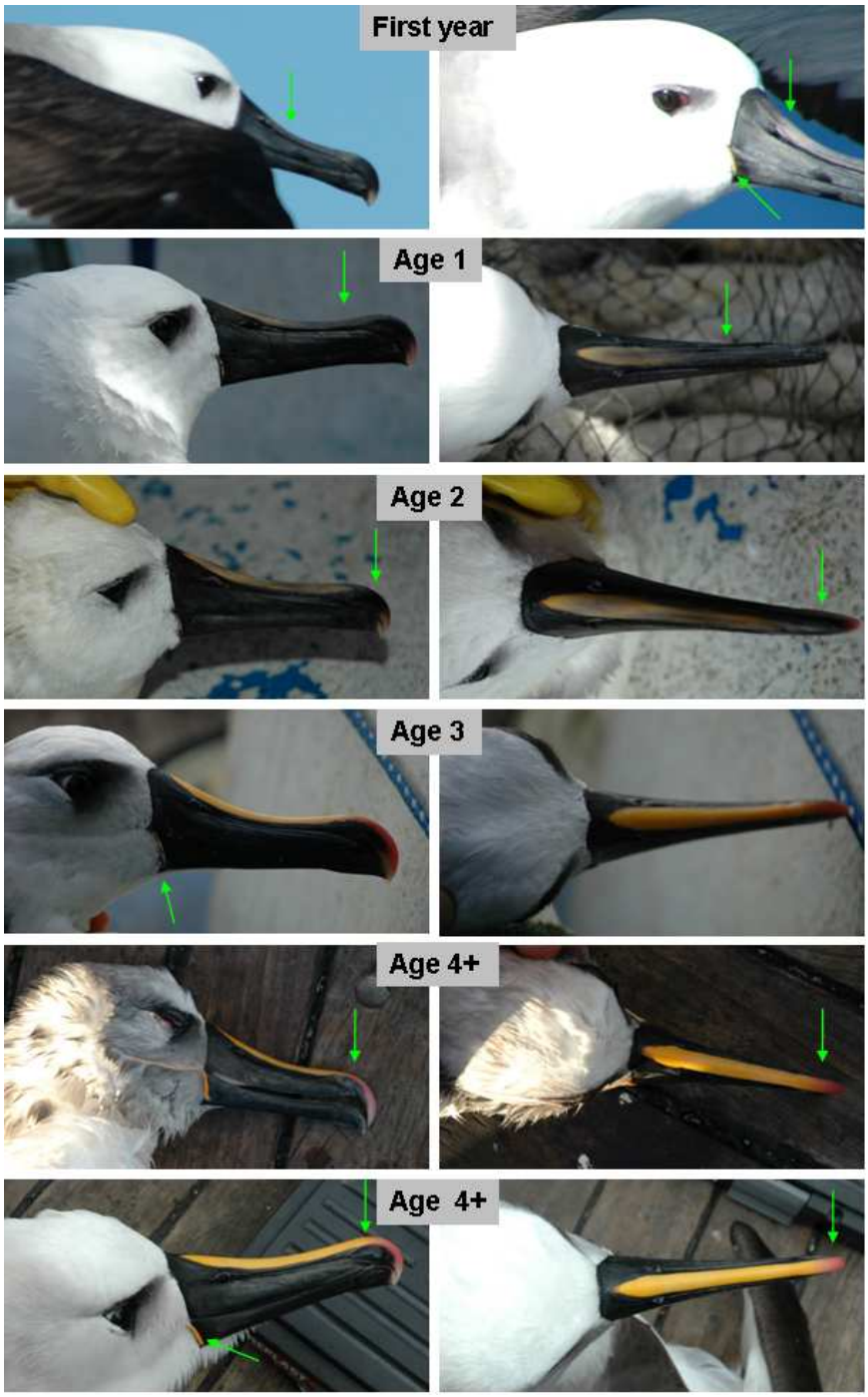

Figure 3.1. Changes in bill colour of the Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*) captured in 2006 and 2007 in the southwestern Atlantic Ocean. Pictures on both columns were taken from the same individual. Arrows indicate diagnostic characteristics for each age. **First year** (recently fledged), early June – no wing or tail moult; **Age 1**, April - no wing moult, but some tail, head, dorsum and ventral moult; **Age 2**, February – final wing moult. Tail and contour moult occurs; **Age 3**, early June – finishing wing, tail and contour moult, while breeders are in the middle of moult. Apart from moult, differs of adult breeder by the pale vertical bar at the lower mandible; **Age 4+** (Adult). No moult, February, enlarged testis and brood patch of this bird was in regression.

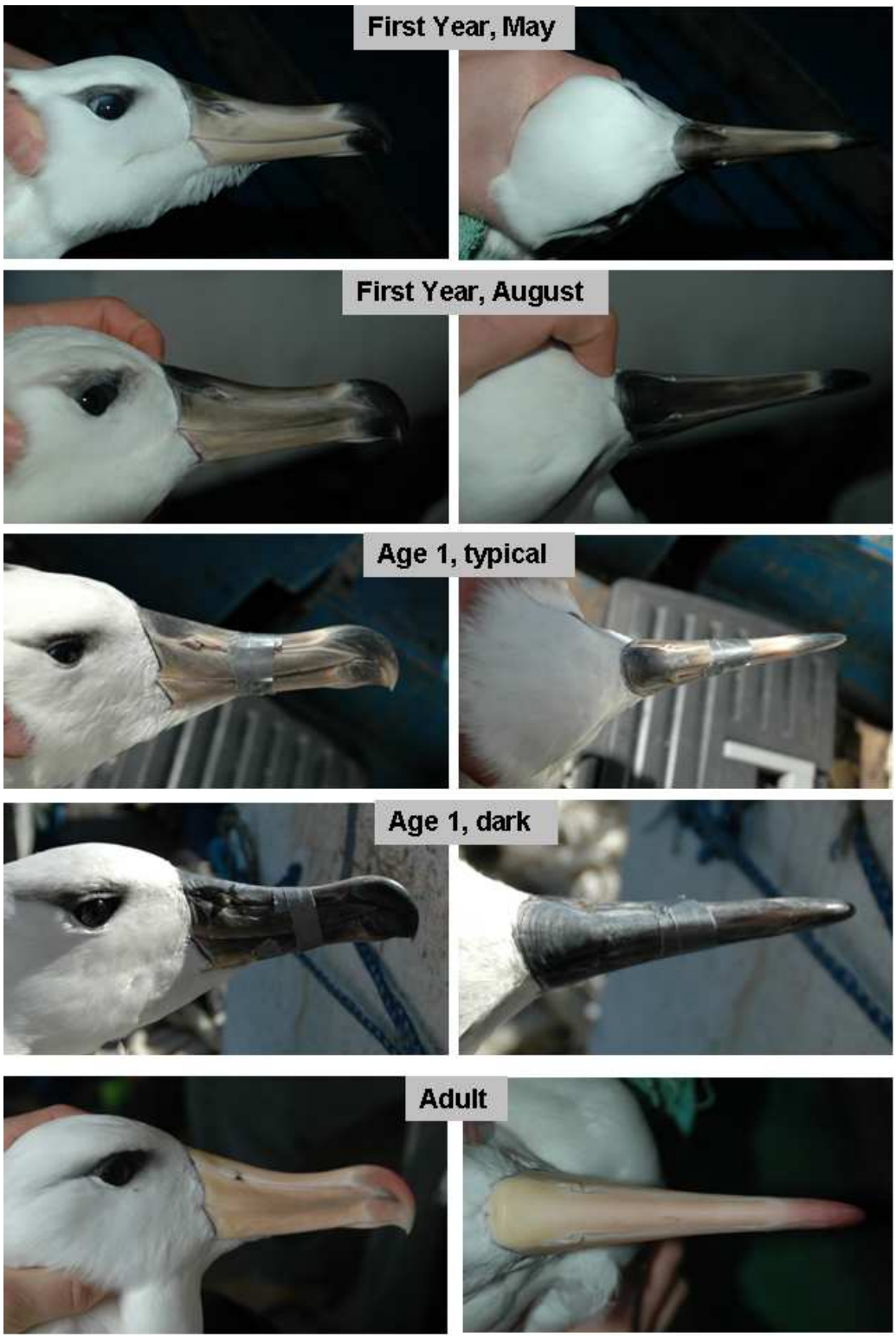

Figure 3.2. Variation in bill colour in Black-browed Albatross (*Thalassarche melanophris*) of different ages captured in the southwestern Atlantic Ocean. Note the pale bill in recently fledged birds captured in May and August. 'Age 1' birds with typical bill and one bird with wholly black bill are suspected to be originated from different colonies.

Chapter 4

EFFECTS OF PRESERVATION METHODS ON STABLE ISOTOPES SIGNATURES IN BIRD TISSUES

Bugoni L, McGill RAR, Furness RW (2008d) Effects of preservation methods on stable isotopes signatures in bird tissues. ***Rapid Communications in Mass Spectrometry*** 22(16): 2457-2462.

ABSTRACT

Increasing use is being made of stable isotopes as indicators of habitat use and trophic ecology of animals. Preservation of tissues can alter stable isotope signatures. We investigated the effects of addition of ethanol and NaCl solution (hereafter "salt"), and of freezing and drying on carbon and nitrogen isotopic values in blood of the Spectacled Petrel *Procellaria conspicillata*, and compared these with simultaneously growing feathers. The mean $\delta^{13}C$ values of blood preserved in ethanol was significantly higher, and of blood preserved in salt was significantly lower than that of dried or frozen samples. $\delta^{13}C$ values in ethanol showed high variation according to brand and batch and could account for the differences found in $\delta^{13}C$ ratios in ethanol-preserved blood samples. Mean $\delta^{13}C$ and $\delta^{15}N$ values in growing feathers were higher than in blood, suggesting tissue-specific fractionation. We conclude that different methods of preserving tissues such as blood may bias stable isotope values, and urge researchers to consider this issue. Air drying is proposed as a practical and unbiased method for blood preservation in field situations where freezing is not a practical option, and a mathematical approach is suggested to permit comparison between studies using different preservation methods or tissues.

INTRODUCTION

Stable isotopes of carbon and nitrogen in bird tissues have been increasingly used in a range of ecological studies (Kelly 2000; Hobson 2005a; Barrett *et al.* 2007). Ecological applications of the stable isotope technique include analysis of food-web structure (Forero *et al.* 2004; Aberle & Malzahn 2007), sexual or age-related segregation (Forero *et al.* 2005), contribution of nutrients from different sources such as marine versus freshwater environments (Bearhop *et al.* 1999), impact of human activities (Bearhop *et al.* 2001), habitat use and dispersion (Bearhop *et al.* 2003; Barrett *et al.* 2007), latitudinal differences in distribution of foraging or wintering areas (Quillfeldt *et al.* 2005; Cherel *et al.* 2007; Gómez-Díaz & González-Solís 2007). The method is based on the fact that stable isotopes in prey frequently have distinct values, and are transferred from prey to predators in a predictable manner (Kelly 2000). Unlike traditional methods (e.g. regurgitations, pellets, stomach flushing) that provide a snapshot of the predator's diet, stable isotopes in animal tissues integrate dietary information over space and time, ranging from days to months (Barrett *et al.* 2007).

For bird studies, muscle and bone collagen were preferred tissues for stable isotopes during early development of the techniques (Kelly 2000), but feather and blood are currently the preferred tissues because they can be sampled non-destructively and the individual can be re-sampled several times (Bearhop *et al.* 2002). Thus the techniques could also be used for studies involving endangered species where lethal methods would raise ethical questions. Feathers are generally preserved dry, with no addition of

preservative at this stage, but prior to isotope analysis are sometimes washed with substances containing carbon such as chloroform, methanol, ether or a mixture of those (Cherel *et al.* 2006; Hedd & Montevecchi 2006; Becker *et al.* 2007) under the assumption that subsequent air drying removes added solvents. Conversely, a range of methods has been used to preserve wet tissues for stable isotope analysis. Muscle of fish, amphibians and small reptiles for historical collections is usually fixed in formalin and stored in 70% ethanol (Arrington & Winemiller 2002; Araújo *et al.* 2007) whereas blood is preserved in ethanol, in heparinised solution, frozen or dried. There is no consensus on the best method for preservation, and some studies suggest that preservation methods alter stable isotope ratios whereas other studies failed to detect any difference caused by preservation (Sarakinos *et al.* 2002; Kelly *et al.* 2006). Despite controversies and a range of studies detecting changes associating with preservation methods, studies measuring stable isotopes in birds frequently continue to make use of preservatives that contain the isotope to be analysed, e.g. ethanol or heparin for analysis of carbon (Cherel & Hobson 2007; Williams *et al.* 2007). In addition, most studies do not report potential errors due to preservatives, and a few even do not mention the blood preservation method. The assumption that preservation effects are negligible, even when using a fixative which contains carbon, apparently still persists in the ornithological literature (e.g. Cherel *et al.* 2007; Haramis *et al.* 2007; Cherel 2008; Harding *et al.* 2008) probably because it was previously shown that ethanol does not change the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signature of preserved blood of captive Japanese quail *Coturnix japonica* fed on commercial ration (Hobson *et al.* 1997). But studies from other taxa have shown clear effects of preservation methods on stable isotope ratios, e.g. in fish tissues (Sarakinos *et al.* 2002; Sweeting *et al.* 2004; Kelly *et al.* 2006) and this question has yet to be fully addressed in wild birds. Furthermore, values of nitrogen in formalin from three different brands showed different isotopic signatures (Edwards *et al.* 2002), but similar comparisons between batches of ethanol have not yet been made, despite a clear need for that (Sweeting *et al.* 2004).

In the present study we investigate the effect of preservation methods on carbon and nitrogen stable isotope values in whole blood of Spectacled Petrel (*Procellaria conspicillata*) and propose a simple regression approach to correct for the effects of different preservation methods and tissues. Mathematical normalization of data has been applied to the problem of samples with high and variable lipid content, which considerably affects the $\delta^{13}C$ and $\delta^{15}N$ values (Post *et al.* 2007; Logan & Lutcavage 2008), or for the effects of preservatives in fish tissues (Kelly *et al.* 2006) but the determination of correction factors has not been previously addressed in bird blood where different preservation methods are used. We were particularly interested in methods that can be used in a range of situations and field conditions, which frequently occur in remote study sites, such as onboard fishing vessels at sea or on remote oceanic

islands. We also addressed the effects of preservatives that contain carbon (i.e. ethanol) in comparison with a carbon-free preservative (i.e. NaCl), and measured isotope signatures in different brands and batches of ethanol.

MATERIAL AND METHODS

Study species

Spectacled Petrel is a pelagic seabird endemic to the Tristan da Cunha Archipelago in the South Atlantic Ocean (Ryan *et al.* 2006). It is a medium-sized petrel (1.3 kg, Ryan 1998) with at sea distribution mainly between 25 and 40°S (Enticott & O'Connell 1985), but concentrated in deep and warm waters of the Southwestern Atlantic Ocean, off southern Brazil and Uruguay (Neves *et al.* 2006b). The species is the most common seabird following pelagic longliners off the Brazilian coast throughout the year (Neves *et al.* 2006b; Bugoni *et al.* 2008a, Chapter 9) feeding on baits and offal discarded by vessels, and non-negligible numbers are killed by pelagic longline fisheries (Bugoni *et al.* 2008a) and a range of other hook-and-line fisheries (Bugoni *et al.* 2008c, Chapter 10).

Sampling methods and preservation

Twenty-one Spectacled Petrels were sampled over the continental shelf in southern Brazil, in February 2006. Birds scavenging for offal and baits were attracted close to the vessel and captured using a cast net (Bugoni *et al.* 2008b, Chapter 2). Birds were individually marked using metal rings to avoid re-sampling the same individual. Needles and syringes were used to collect 1 mL of blood from the tarsal vein of birds. Sub samples composed of two or three drops of whole blood were stored in different ways for testing for the effects of differences in preservation methods on carbon and nitrogen isotope ratios. Treatments (preservations methods) were: 1) blood in 1.5 mL of absolute Merck® ethanol; 2) blood in 1.5 mL of saturated salt solution ($NaCl/H_2O$); 3) drops of blood placed in a glass microscope slide (previously cleaned of possible surface contaminants using cotton), dried in air, scraped and stored in plastic tubes; 4) blood frozen in the facilities available onboard the vessel. In addition, five to eight partially grown body feathers, still with blood in the calamus (shaft) were collected, the blood in the calamus removed, and the feathers placed in sealed plastic bags. Growing feathers are tissues with similar age to whole blood, i.e. a few weeks, and assumed to be formed by the same pool of nutrients circulating in the blood. All samples were transported under permit to University of Glasgow where they were prepared for analysis. During transport, frozen samples were maintained in a Styrofoam box with ice replaced frequently.

Carbon isotope values in the absolute ethanol from two bottles of different batches from Merck® (Darmstadt, Germany), one of which was also used for blood preservation, and from a bottle from Fisher Scientific (Loughborough, UK) were analysed. As $\delta^{13}C$ values between brands were different and close to values expected for C_3 and C_4 plants (see

Results and Discussion), we contacted manufacturers asking for methods of ethanol production, which informed that both brands and batches were chemically produced in laboratory, rather than derived from crops.

Sample analysis

All blood samples were freeze-dried, ground and homogenised. Feathers were washed five times in distilled water, dried in an oven for two hours under constant temperature of 70ºC and cut with scissors. Fragments of several feathers were used. Samples of approximately 0.65 mg of blood or 0.70 mg of feathers were weighed, placed in tin capsules (4x6 mm) and analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech Elemental Analyser (Costech ECS 4010, Milan, Italy) linked to a Thermo Finnigan Delta Plus XP Mass Spectrometer (Thermo Finnigan, Bremen, Germany). Three laboratory standards were analysed for every 10 unknown samples in each analytical sequence, allowing instrument drift to be corrected. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards Pee Dee Belemnite limestone (carbon) and atmospheric air (nitrogen), according to the following equation

$$\delta X = \left[\left(R_{sample} / R_{standard}\right) - 1\right] \times 1000 \qquad \text{(equation 4.1)}$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Samples were shuffled in the analytical sequence according to different tissues, individuals, and preservation methods to minimize drift with time (Jardine & Cunjak 2005). For ethanol samples, an aliquot of 1.5 µL was pipetted into smooth wall tin capsules (5x2 mm) model D4042 (Elemental Micro Analysis Ltd., Okehampton, UK) and were sealed using small wire cutters (side cutters) and analysed as above.

Data analysis

$\delta^{15}N$ and $\delta^{13}C$ values for each preservation method were compared by one-way ANOVA considering individual birds as random effects, for both nitrogen and carbon. $\delta^{13}C$ values were transformed $\log(n \times -1)$, thus accounting for negative values, and aiming to achieve normality (tested by Kolmogorov-Smirnov test) and homoscedasticy of residuals (tested by Levene's test). For post hoc comparisons Tukey HSD test was used. Correlation coefficients between values found for preservation methods were calculated and Bonferroni correction applied for multiple comparisons. Values were reported as mean ± one standard deviation. Measurement precision of both $\delta^{15}N$ and $\delta^{13}C$ was better than 0.3‰.

RESULTS

Carbon

Mean $\delta^{13}C$ in blood varied from -17.1‰ to -18.4‰ and in growing feathers was -16.0‰ (Table 4.1, Fig. 4.1). Random effects were significant ($F_{20, 75}$ = 13.6 P < 0.001) indicating consistent differences among individuals. Preservation methods affected the carbon signatures ($F_{4, 75}$ = 174.3, P < 0.001, Fig. 4.1). Carbon isotope ratios were different for all treatments (P < 0.001), except between dry and frozen samples (P = 0.34). Ethanol preserved samples were ^{13}C enriched and simultaneously growing feathers considerably enriched in comparison with dry and frozen blood samples (Fig. 4.1). In contrast, NaCl preserved samples were ^{13}C depleted, and values were more variable than for other preservation methods (see SD values in Table 4.1), despite non significant differences in variance of untransformed data between treatments (Levene's test = 0.69, P = 0.6).

Carbon isotope ratios in ethanol differed, showing very different values according to batch and manufacturer (Table 4.1). Merck ethanol used for blood preservation in this study had higher $\delta^{13}C$ values (-12.1‰) than the range of individual values found in blood samples (-22.5 to -16.3‰), whereas Fisher ethanol had lower $\delta^{13}C$ value (-30.0‰). Another batch of Merck ethanol had intermediate values of -24.3‰.

Correlations in $\delta^{13}C$ values in blood preserved using the different methods, and feathers, among individual birds were strong, despite the effect of treatment (Table 4.2).

Nitrogen

Mean values of $\delta^{15}N$ in blood varied from 13.6‰ to 14.0‰ according to different treatments and were significantly more enriched in growing feathers (15.5‰, Table 4.1, Fig. 4.2). Variation among treatments was also found in a similar way as found for carbon ($F_{4, 75}$ = 174.3, P < 0.001, Fig. 4.2). There were also differences between individuals (random effects $F_{20, 75}$ = 13.6, P < 0.001). The main distinction in comparison with carbon was that nitrogen isotope ratios in samples preserved in ethanol were not significantly different from those in dry and frozen samples (P = 0.11 and P = 0.10, respectively), a predicted result since ethanol does not contain nitrogen. NaCl preserved samples were ^{15}N depleted, but values showed similar variability to other treatments (Table 4.1, Fig. 4.2).

Correlations in $\delta^{15}N$ values in blood preserved using the different methods, and feathers, among individual birds were high, despite the effect of treatment (Table 4.3).

Mathematical correction

It is shown in the present study (Tables 4.2 & 4.3) that there is strong correlation, across individual birds, of isotopic values in two different tissues preserved in different ways for

both δ^{15}N and δ^{13}C. This property of data allows data to be mathematically corrected. For example, using the regression equation

$$\delta^{13}\mathrm{C}_{\mathrm{Frozen}} = -7.794 + 0.6176\delta^{13}\mathrm{C}_{\mathrm{Feather}} \quad \text{(equation 4.2)}$$

obtained from the correlation between δ^{13}C in growing feather and frozen blood in Spectacled Petrels, one could remove the effect of different tissues and make values obtained by the analysis of different tissues comparable. Similarly, effects of preservation methods can be corrected. For instance, to remove the effect caused by ethanol on δ^{13}C values in blood, one could use the equation

$$\delta^{13}\mathrm{C}_{\mathrm{Frozen}} = -3.395 + 0.8371\delta^{13}\mathrm{C}_{\mathrm{Ethanol}} \quad \text{(equation 4.3)}$$

which related δ^{13}C values in frozen blood with δ^{13}C values in ethanol preserved blood.

DISCUSSION

Ecological questions addressed by stable isotope analysis often require detection of changes of less than 2‰ (see Sweeting *et al.* 2005 and references therein). For example, diet-tissue fractionation of δ^{13}C in trophodynamic studies is assumed to be 0-1‰ per trophic level (Ng *et al.* 2007; Sweeting *et al.* 2007), or smaller among species in studies comparing several species in a community (e.g. Forero *et al.* 2004 and references therein). Thus, variation caused by preservation could influence the interpretation of a biological phenomenon underlying the differences. As we have shown here, differences of this scale could be caused by preservation method (e.g. δ^{13}C mean difference in blood preserved in NaCl vs Ethanol = 1.3‰), different tissues synthesised simultaneously (e.g. mean difference in δ^{15}N in frozen blood vs growing feathers = 1.5‰, see also Cherel *et al.* 2005), or using different brands or batches of the same type of preservative (Merck ethanol vs Fisher ethanol, Table 4.1). It is also noteworthy that although we freeze-dried blood samples prior to isotope analysis, the results show clearly that this procedure did not remove the influence of ethanol on the carbon isotope signal. These examples emphasize the need to be consistent, which means being aware of the isotopes in the preservatives and using the same brand and/or batch of preservative (first mixing several bottles if large volumes are required). Lack of information on preservatives used may preclude accurate comparisons across different studies.

In studies of seabirds using stable isotopes, samples are frequently collected in different islands or colonies and preservation methods can be chosen based on practicality in field situations, sometimes resulting in the preservation of blood in one way and potential prey such as fish, squid, and crustaceans in another. Additional problems may occur

using a standard preservative, e.g. ethanol, without taking into account the carbon signature in the preservative. By using ethanol from different crops, such as C_3 and C_4 plants, or different brands, the changes to the stable isotope value of the sample caused by the preservative could be as great as the biological differences under investigation.

A decrease of $\delta^{13}C$ (by 0.7‰) and $\delta^{15}N$ (by 0.6‰) in samples preserved in NaCl solution in comparison with frozen blood, and greater variance in values from samples, parallel results reported for the use of mercury chloride ($HgCl_2$) to preserve fish and crustacean tissues (Bosley & Wainright 1999). Mechanical cell destruction and loss of cytosol or metabolic shifts may occur. These effects were suggested as possible causes of decrease in carbon and nitrogen values in bulk frozen macrozoobenthos (Dannheim *et al.* 2007). From a fieldwork perspective, NaCl is an easy method to use to preserve a range of tissues and is applicable in remote field sites, but it greatly affected results for carbon and nitrogen isotopes and resulted in greater sample variability. In contrast to our results, a previous study (Arrington & Winemiller 2002) suggested that salt is as good as freezing for the preservation of fish muscle for $\delta^{13}C$, but significantly altered values of $\delta^{15}N$.

Using ethanol to preserve blood significantly increased $\delta^{13}C$ (0.6‰), but caused a non-significant increase in $\delta^{15}N$ (0.3‰). A similar enrichment in $\delta^{13}C$ of 0.7 to 1.5‰ was also detected in fish, octopus and kelp (Kaehler & Pakhomov 2001), fish muscle and liver (Sweeting *et al.* 2004) and fish and mollusc muscle (Sarakinos *et al.* 2002). For blood samples preserved in ethanol, mechanical cell destruction could potentially occurs, with effects similar to those suggested above for blood preserved in NaCl. This process resulted in lower carbon isotope values in blood samples, but the $\delta^{13}C$ value of the ethanol itself is much higher. Carbon isotope values of blood preserved in ethanol were higher than for the dried and frozen samples, which suggests that some carbon present in the ethanol was incorporated into the sample. It is suggested that ethanol alters the carbon signature by acting as a solvent of compounds which have lower carbon values such as lipids (Sweeting *et al.* 2004; Dannheim *et al.* 2007). Blood is a lipid-poor tissue, so this is unlikely to be the case in the present study. Moreover, the $\delta^{13}C$ value in ethanol used previously to preserve fish tissues (Sweeting *et al.* 2004) was -28.72‰ and this also resulted in ^{13}C enrichment in samples, although this $\delta^{13}C$ value in ethanol was lower than $\delta^{13}C$ value in samples. These authors suggested that ethanol could be used in low fat tissues, but our results have shown that ethanol does affect $\delta^{13}C$ values in lipid poor tissues such as blood.

Drying blood at ambient air temperature is also a valuable method of blood preservation in remote field sites because only two or three drops of blood are enough for stable

isotope analysis. They can be dried on a clean glass slide, scraped off once dry and stored in a tube. This method was as effective as freezing samples for $\delta^{13}C$ and $\delta^{15}N$ in the current study and dispenses with the need for a power supply for freezing and sample storage.

There is no consensus regarding sample preservation and preparation for stable isotopes analysis. Some aspects such as the choice of tissue are hard to standardize because different tissues provide information on diet at different time scales. However preservation methods could be standardized in order to improve the comparability of data across studies. Differences between feather and blood isotopic signatures are due to different protein turnover rates and metabolic processes (Hobson & Clark 1992; Vanderklift & Ponsard 2003; Cherel & Hobson 2007), thus different tissues are not directly comparable (Cherel & Hobson 2007). As we found in this study, feathers usually have enriched ^{13}C and ^{15}N values than blood (Cherel & Hobson 2007). However, values in different tissues could be arithmetically corrected if their relationships are first determined, as the equations based on regressions shown in Results. Mathematical corrections could also be applied to $\delta^{15}N$ where shifts are consistent as demonstrated in this study (Table 4.3). Moreover, empirical mathematical corrections should be considered on a case-specific basis, because they can vary according to tissue, species, isotope and preservation method, preservative brand and/or batch, or no correlation maybe found (Sweeting *et al.* 2004), so no correction is possible. Thus, despite the promising nature of the method proposed here for inter-tissue comparison, more detailed studies are required in order to test their validity under different conditions. Understanding the effects of preservatives and fixatives in different tissues, with the simultaneous establishment of correction factors, could greatly aid the use of isotope ratios in preserved specimens from museums (Arrington & Winemiller 2002; Edwards *et al.* 2002). Lipid composition in fish tissues is variable and shown to influence the isotopic values in preserved samples, which complicates the determination of a non-species-specific correction (Kelly *et al.* 2006; Sweeting *et al.* 2006). Despite variations in lipid composition in bird blood according to absorptive (Bearhop *et al.* 2002), reproductive and nutritional states (Hobson *et al.* 1993), large variations in lipid content are not expected and a general correction factor is a further possibility.

Carbon isotope ratios in ethanol measured in the present study were distinguished by a difference of 17.9‰ between two different producers, with values characteristic of C_3 and C_4 plants (Fisher and Merck, respectively) and two different batches from the same manufacturer showed a difference of 12.2‰ (Table 4.1). However, manufacturers informed the authors that both were derived in laboratory rather than produced from crops, thus the measured carbon isotope difference was caused by some chemical procedure or variation in the carbon present in the raw material used. Differences in the

signatures of C_3 and C_4 plants has been widely used in ecological studies (Kelly 2000), as well as for distinguishing ethanol produced from C_3 (e.g. barley, sugar beet) or C_4 plants (e.g. corn, sugar cane; Ishida-Fujii *et al.* 2005). Isotopic differences in ethanol are largely used to determine the authenticity of food, beverages, cosmetics and other products (Ishida-Fujii *et al.* 2005). Because differences in ethanol $\delta^{13}C$ values vary widely according to the crop (from -10.7‰ to -27.7‰ in Ishida-Fujii *et al.* 2005), or the brand (-12.1‰ and -30.0‰, this study), ethanol can potentially introduce significant errors in the isotopic values of blood samples. We did not test the effect of different ethanol manufacturers on blood, but because ^{13}C enriched ethanol increased the $\delta^{13}C$ value in blood, we can predict that ^{13}C depleted ethanol will probably cause a decrease in $\delta^{13}C$ values in bird blood. $\delta^{13}C$ in fish muscle was shown to have isotopic values altered by ^{13}C depleted formalin used as fixative from three different manufacturers and delta values ranging from -37.8 to -52.5‰ (Edwards *et al.* 2002). These authors demonstrated that the magnitude of carbon isotopic change in tissues can depend on the isotopic composition in the fixative. To address the problem of variation in seabird studies due to the use of different preservation methods or chemical preservatives (or the same preservative with different isotopic signatures) within prey types or between prey and bird tissues we strongly recommend improve standardization of sample preparation. In particular the isotope value of the preservation agent should be measured or known, e.g. $\delta^{13}C$ analysis for ethanol was a simple procedure in this study, and ethanol aliquots were run in parallel with tissue samples (Sweeting *et al.* 2004; Ishida-Fujii *et al.* 2005).

CONCLUDING REMARKS

In general, different studies agree that freezing samples causes little or no effect on organic tissues for stable isotope analysis (Sweeting *et al.* 2004; Post *et al.* 2007, but see Feuchtmayr & Grey 2003), in agreement with our results on Spectacled Petrel blood. Freezing samples is a good option in some field situations and when portable freezer or dry ice is available. When possible, air drying could also be a practical, inexpensive and reliable method for blood preservation. Drying could also be feasible in some places by using an oven/grill or similar equipment that does not produce fire and smoke.

From results presented here, freezing and drying preservation methods are the recommended options for $\delta^{13}C$ and $\delta^{15}N$ studies, when logistics allow the use of these techniques. However, due to the practicality of ethanol as fixative and preservative, particularly for muscle, their utility in $\delta^{13}C$ studies could be further benefited by mathematic corrections like those suggested here, while its effect on $\delta^{15}N$ appears to be negligible. The development of species-specific correction factors is recommended until the effects of preservation methods across species and tissues are better understood.

Table 4.1. Mean ± one standard deviation (minimum and maximum) of values of carbon and nitrogen stable isotope ratios in blood and growing feathers of Spectacled Petrel *Procellaria conspicillata* sampled in southern Brazil and preserved using different methods.

Sample (sample size)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
BLOOD		
Dried (21)	-17.7 ± 0.7 (-20.5 to -16.7)	14.0 ± 0.8 (12.3 to 15.3)
Frozen (20)	-17.7 ± 0.5 (-18.4 to -16.7)	14.0 ± 0.8 (12.3 to 15.4)
Absolute ethanol (21)	-17.1 ± 0.4 (-17.8 to -16.3)	14.3 ± 0.8 (12.4 to 15.7)
NaCl saturated solution (18)	-18.4 ± 1.2 (-22.5 to -16.7)	13.6 ± 0.7 (12.2 to 14.8)
Feathers (21)	-16.0 ± 0.6 (-16.9 to -14.8)	15.5 ± 1.0 (13.8 to 17.1)
Absolute Ethanol Merck Batch 1 (10)	-12.1 ± 0.1 (-12.2 to -12.0)	--
Absolute Ethanol Merck Batch 2 (5)	-24.3 ± 0.1 (-24.5 to -24.2)	--
Absolute Ethanol Fisher (10)	-30.0 ± 0.1 (-30.0 to -29.9)	--

Table 4.2. Correlation coefficients of carbon isotope ratios across individual birds in blood preserved in different ways and simultaneously growing feathers of Spectacled Petrels *Procellaria conspicillata*. All values are significant at $P < 0.005$ (Bonferroni correction for multiple comparisons applied). [a]Non-significant after Bonferroni correction ($P = 0.0056$).

Treatments (sample size)	Blood dried	Blood frozen	Blood in absolute ethanol	Blood in NaCl saturated solution
Blood frozen (n = 20)	0.66			
Blood in absolute ethanol (n = 21)	0.88	0.75		
Blood in NaCl saturated solution (n = 17)	0.79	0.64[a]	0.85	
Growing feathers (n = 21)	0.71	0.75	0.81	0.69

Table 4.3. Correlation coefficients of nitrogen isotope ratios across individual birds in blood preserved in different ways and simultaneously growing feathers of Spectacled Petrels *Procellaria conspicillata*. All values are significant at $P < 0.005$ (Bonferroni correction for multiple comparisons applied).

Treatments (sample size)	Blood dried	Blood frozen	Blood in absolute ethanol	Blood in NaCl saturated solution
Blood frozen (n = 20)	0.87			
Blood in absolute ethanol (n = 21)	0.90	0.91		
Blood in NaCl saturated solution (n = 17)	0.79	0.96	0.91	
Growing feathers (n = 21)	0.71	0.85	0.82	0.84

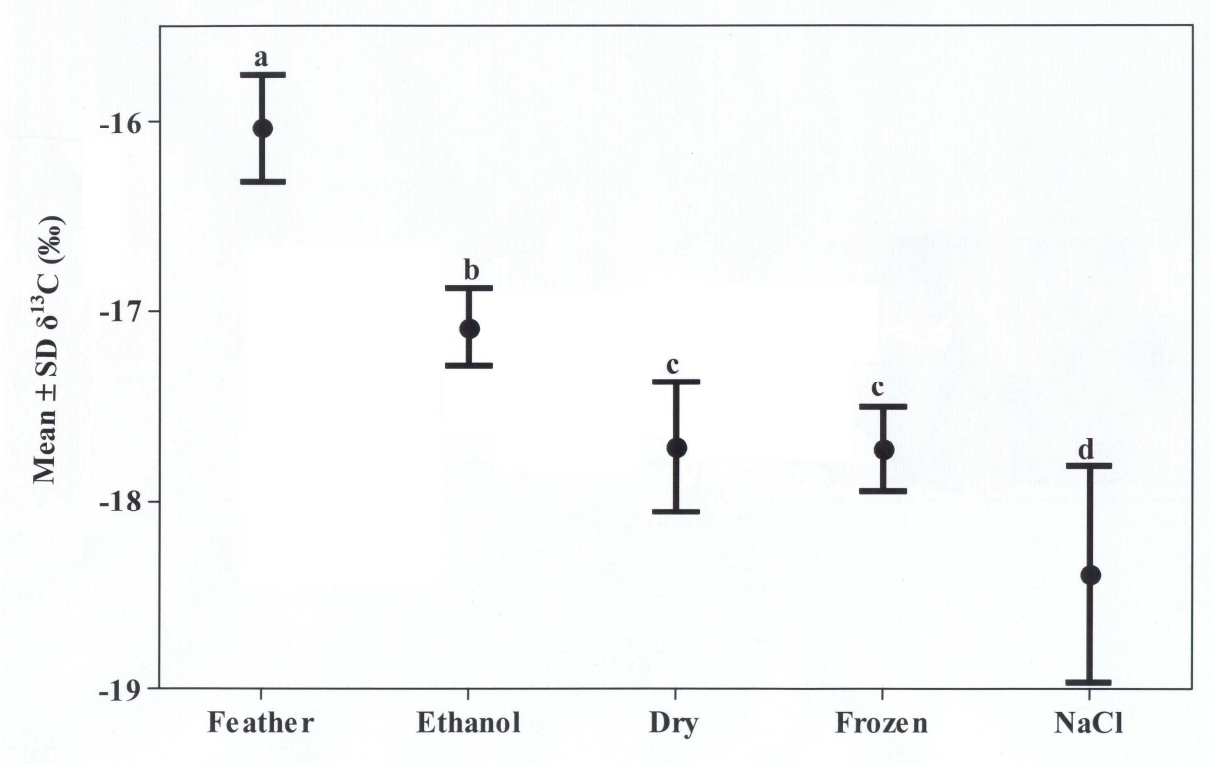

Figure 4.1. Mean carbon isotope ratios (± 1 SD) in blood preserved in different ways and in simultaneously growing feathers of Spectacled Petrels *Procellaria conspicillata* wintering off Brazil. Different letters indicates significant differences at $P < 0.05$ (Tukey HSD test).

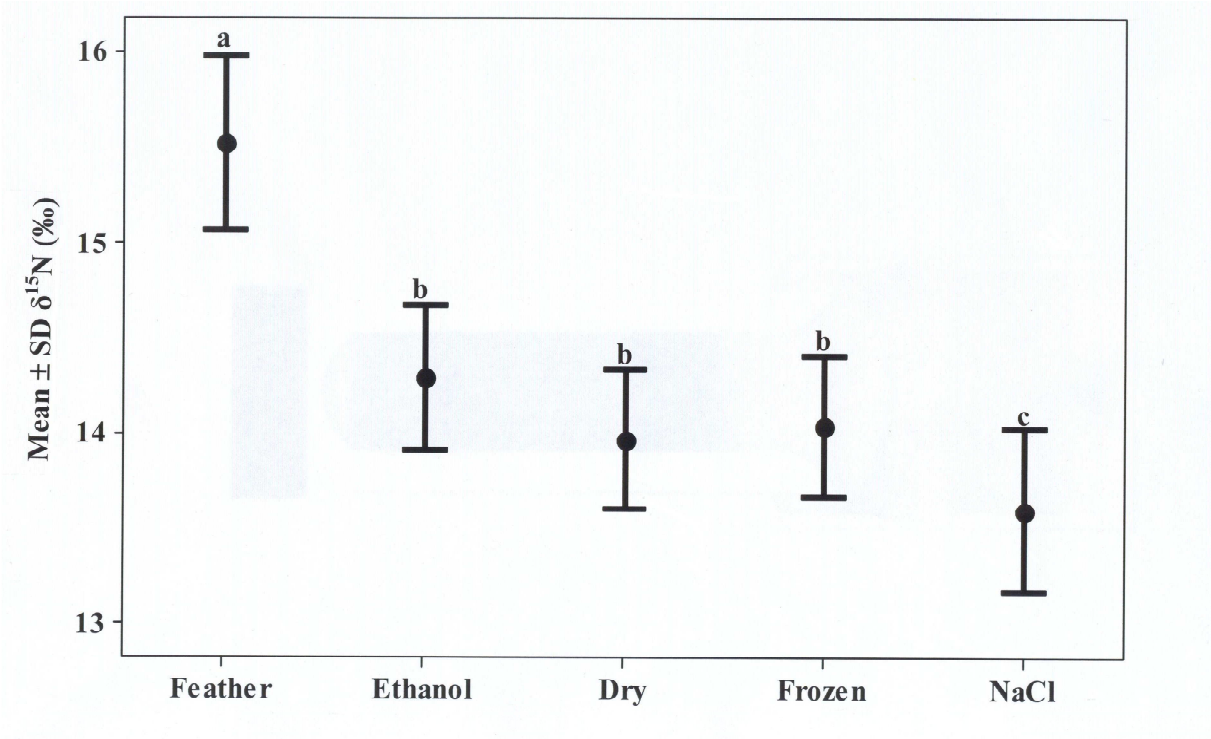

Figure 4.2. Mean nitrogen isotope ratios (± 1 SD) in blood preserved in different ways and in simultaneously growing feathers of Spectacled Petrels *Procellaria conspicillata* wintering off Brazil. Different letters indicates significant differences at $P < 0.05$ (Tukey HSD test).

Chapter 5

PATTERNS OF MOULT AND BREEDING-MOULT OVERLAP IN ALBATROSSES, PETRELS AND SHEARWATERS

Bugoni L, Furness RW. Patterns of moult and breeding-moult overlap in albatrosses, petrels and shearwaters. **Under review**

ABSTRACT

Patterns of moult of four albatross and ten petrel and shearwater species were described for primary, tail and contour traits, and in relation to breeding period. Birds were sampled at sea off the Brazilian coast from February to September. Procellariiformes appear to have two distinct moulting strategies: petrels and shearwaters have complete annual moult, start to moult during the breeding period, and replace several primaries and tail feathers at one time, whereas albatrosses undergo long moulting cycles, replace less feathers at once, and suspend the wing moult during breeding periods, which could limit flight efficiency. Primary moult starting at P2 rather than P1 was demonstrated to be a common feature among Procellariiformes, with important implications for studies of stable isotopes, trace elements and pollutants in feathers. Brazilian waters were shown to be an important moult area for immature Great Shearwaters (*Puffinus gravis*). Overlap between moulting and breeding is demonstrated to be common with tail and contour feathers, but limited in wing, which suggests that flight constraint in long distance foragers rather than nutritional and energetic limitations is the ultimate factor determining primary moult timing.

INTRODUCTION

A general paradigm is that birds avoid overlap of highly demanding activities, such as breeding and moulting (Payne 1972; Murphy & King 1991). Among Procellariiformes, the albatrosses do not undertake primary moult while breeding (Furness 1988; Prince *et al.* 1993; Edwards 2008), whereas high latitude petrels usually start moult during breeding (Beck 1969; Hunter 1984a; Barbraud & Chastel 1998), a strategy to benefit from seasonally abundant polar food sources (Hunter 1984a; Barbraud & Chastel 1998). Tropical/subtropical species which perform long transequatorial migrations could also start primary moult during breeding, as demonstrated for Cory's Shearwater (*Calonectris diomedea*) breeding in the Azores (Monteiro & Furness 1996), while Great Shearwaters (*Puffinus gravis*) breeding in Tristan da Cunha islands delay moult until arrival in the northwest Atlantic Ocean (Stresemann & Stresemann 1970; Brown 1988). Moreover, closely related species can differ markedly as, for instance, the Southern Fulmar (*Fulmarus glacialoides*) which starts moulting during incubation, while the Northern Fulmar (*Fulmarus glacialis*) does not initiate primary moult before chick-rearing (Barbraud & Chastel 1998; Allard *et al.* 2008). An alternative hypothesis has been proposed, that impairment in flight performance rather than energetic constraint may be the selective advantage of avoiding overlap in moult and breeding, particularly during demanding periods such as while feeding chicks (Beck 1969; Weimerskirch 1991). If energetic constraint is the primary limiting factor for moulting during breeding and if the cost for replacing any feather of similar size is energetically equivalent one could expect that birds would avoid moulting tail and body feathers as much as they avoid primary moult. For example, body feathers correspond to 75-80% of total feather weight in Cape

Petrels (*Daption capense*) (Beck 1969), thus their synthesis is energetically at least as costly as wing flight feathers. Unfortunately, studies of moult usually rely mostly or exclusively on primary (flight) feathers, which preclude disentangling these different hypotheses. Furthermore, studies of moult in Procellariiformes have almost all been carried out on breeding grounds, when moult is not active, or cover a limited moulting period (Furness 1988; Prince *et al.* 1993; Allard *et al.* 2008), rely on limited numbers of specimens from museums (Marchant & Higgins 1990; Bridge 2006), make use of opportunistic birds washed ashore, or incidentally killed in fisheries (Cooper *et al.* 1991; Ryan 1999; Edwards & Rohwer 2005; Bugoni *et al.* 2007; Edwards 2008), or observations of moult of birds at a distance, which only allow the identification of large gaps in wing and tail moult (Watson 1971; Huettmann & Diamond 2000). Despite different drawbacks, these methods have provided virtually all information on moult of Procellariiformes.

Furness (1988) described the wing (primary) moult of breeding Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*) at Gough Island and suggested multiple moulting waves, suspended moult during the breeding period, and up to three years for completion of primary feather replacement, while Prince *et al.* (1993) reported that Black-browed (*T. melanophris*) and Gray-headed (*T. chrysostoma*) Albatrosses from South Georgia had descendant moult of outer and ascendant moult of inner primary feathers in alternate years. More complex but similar moult occurs in Wandering Albatrosses (*Diomedea exulans*) from the same place (Prince *et al.* 1997b). All these studies were carried out on breeding grounds. For two Pacific Ocean albatrosses, the Laysan (*Phoebastria immutabilis*) and the Black-footed (*P. nigripes*) Albatrosses incidentally captured in the driftnet fishery, moult also proceeded in opposite directions (descendant in outer primaries and ascendant for inner), but recommenced from the last feather replaced in the previous year, in a 'wrap-around' pattern (Langston & Rohwer 1995, 1996; Edwards & Rohwer 2005; Edwards 2008). It is generally accepted that Albatrosses (Diomedeidae) take two or more years to replace all primary feathers, with interruptions for breeding, while petrels and shearwaters (Procellariidae) have complete descending moult of primaries annually (Marchant & Higgins 1990). Moult of several petrels has been studied on breeding grounds, made easier because primary moult starts early in the breeding season (Hunter 1984a; Monteiro & Furness 1996; Barbraud & Chastel 1998). As with albatrosses, few accounts are available for petrels during the wintering period, and many species remain to be studied.

Detailed information of moult in seabirds is essential in a range of life-history, energetic and environmental studies (Bridge 2006; Allard *et al.* 2008). For example, chemical analysis of feathers has been used to determine the origin, migration, wintering areas and association with pollutants. These studies use feathers to measure stable isotopes

(Cherel *et al.* 2000; Hedd & Montevecchi 2006; Gladbach *et al.* 2007), trace elements (Gómez-Díaz & González-Solís 2007), or heavy metal burdens (Furness *et al.* 1986; Becker *et al.* 2002). To address such questions on distribution, ecology or pollution it is essential to have a clear understanding of patterns, duration and timing of moult. However, seabirds which moult during the non-breeding period and are truly pelagic, such as albatrosses and petrels, cannot easily be studied at sea. For these species there is, consequently, a general scarcity of detailed studies on moult, and even basic parameters remain unknown or equivocal (Cooper *et al.* 1991; Bridge 2006).

The recent development of a method to capture large numbers of healthy live seabirds at sea (Bugoni *et al.* 2008b, Chapter 2) facilitates the study of moult in seabirds away from breeding grounds. Here we provide comparative data on the moult of four albatross and ten petrel and shearwater species captured at sea in the southwestern Atlantic Ocean, when nonbreeding birds are expected to be in active moult. In addition to wintering birds, we also sampled breeding birds which forage in the area, first year juveniles, immatures and migrating birds crossing the area. Patterns of wing, and the poorly studied tail and contour moult are described and discussed in relation to overlap with breeding activities. We predict that if flight constraint is more important than nutritional/energetic limitations, breeding birds will not moult flight (primary) feathers, but would moult contour and tail feathers. On the other hand, if the limitation is energetic rather than in flight performance, breeding birds would avoid to moult any feather (as energetic cost for feather synthesis is similar) during energetic demanding periods such as breeding.

METHODS

Study area and seabird capture

Birds were captured over the continental shelf and offshore waters in southern Brazil in the region under the influence of the Subtropical Convergence, formed by the meeting of the warm tropical Brazilian Current flowing southward and the cold Falkland (Malvinas) Current flowing northward (Garcia 1998). Work was carried out onboard fishing vessels targeting Tuna (*Thunnus* spp.), Sharks (mainly Blue Shark *Prionace glauca*) and Swordfish (*Xiphias gladius*) and using a range of hook-and-line and pelagic longline fishing methods as described by Bugoni *et al.* (2008a, c). Birds were captured by attracting them close to the vessel using baits and shark liver and using a cast net to catch selected individuals or groups of birds (Bugoni *et al.* 2008b). Captures occurred during six cruises totaling 58 trapping days from February to June 2006 and late July to early September 2007, from 25 to 35ºS. In addition, a few birds incidentally captured by fishing vessels on longline hooks, were sampled from February to June, but not in August-September.

Plumage moult

Moult scores for each of the 10 primary feathers of the right wing (from the inner P1 to the outer P10) and for all tail feathers were recorded according to age and stage of development (Ginn & Melville 1983). For every feather a score from '0' to '5' was given, as follows: 0 - old feather remaining; 1 – old feather missing or new feather in pin; 2 – feather emerging from the sheath up to 1/3 grown; 3 – new feather between 1/3 and 2/3 grown; 4 - new feather >2/3 and with remains of waxy sheath at its base; 5 – new feather fully developed with no waxy sheaths remaining at the base. The vestigial 11th primary feather present in Procellariiformes (Marchant & Higgins 1990) was not scored.

Distinguishing old from fully grown new feathers is sometimes difficult, and was assisted by the color and brightness of the feather plus the appearance of the feather tip. Old feathers are generally paler and with ragged tips (Fig. 5.1). Contour feather moult on head, dorsum and ventral parts was recorded as occurring or not if at least five growing feathers were located. Birds were also examined for the presence of a brood patch. It was assumed that wing moult is symmetrical, which is generally the case for petrels and albatrosses (Furness 1988; Prince *et al.* 1993; Bugoni *et al.* 2007) and so moult scores were recorded only for the right wing. Score values were summed, with overall possible values varying from 0 (all old feathers) to 50 (all recently grown feathers). For the less symmetrical tail, moult scores for all feathers were recorded, with possible summed values up to 60 for species with six pairs of rectrices (e.g. albatrosses, shearwaters, *Procellaria* spp.), up to 70 for Cape Petrel (*Daption capense*) which have seven pairs, and up to 80 for Southern and Northern Giant Petrels (*Macronectes giganteus* and *M. halli*, respectively) which have eight pairs of rectrices (Beck 1969; Marchant & Higgins 1990). A variable number of rectrices was recorded in Southern Fulmar from 6 to 8 pairs (see results). We sexed all birds using PCR amplification of CHD genes using primers 2550F (Fridolfsson & Ellegren 1999) and 2757R (R. Griffiths, umpublished), but because in the samples of albatross there was a large number of juveniles and in the petrels we could not differentiate immatures from adults, we could not identified obvious sex-related differences in moult. However, moult of males and females do not differed in previous studies (Furness 1988; Prince *et al.* 1993; but see Hunter 1984a for breeding Giant Petrels) and so were not considered in the results.

Pictures of bill and general plumage were taken from albatrosses and Giant Petrels for ageing. Giant Petrels change plumage colour from entirely dark brown to pale brown-grayish with age, which was also used to identify juveniles from older birds (Onley & Scofield 2007). Ageing of Black-browed and Atlantic Yellow-nosed Albatrosses was described elsewhere (Chapter 3) based on changes in bill color, and wing moult, which allow age determination of juvenile and immature classes up to six years-old (Prince & Rodwell 1994). The pattern of plumage maturation has been described for Wandering

Albatross by Gibson (1967), which allows ageing birds of known sex even for breeding adults by 5-10 years of accuracy (Prince *et al.* 1997b) and was used here, further benefited because we trapped some ringed birds of known-age. Because Wandering and Tristan (*Diomedea dabbenena*) Albatrosses can not be discriminated at specific level by plumage (Onley & Scofield 2007), we used the discriminant function in Cuthbert *et al.* (2003a) to assign species. After sexing birds using molecular techniques we further confirmed species identification using culmen length (Cuthbert *et al.* 2003a). For Wilson's Storm-petrels (*Oceanites oceanicus*) moult patterns and foot web coloration were used for ageing (Quillfeldt *et al.* 2000).

Age classes of birds are referred to as 'first year juveniles', from fledging to the completion of the first year at sea, when no moulting occurs; 'immature', from the end of the first year to recruitment or when they acquire the plumage of adults. These birds were expected to have started moulting during the breeding period of the species; and 'adults', birds with plumage as adults, irrespective of whether individuals were breeding or not. These criteria were also used for albatrosses, but plumage and bill color were further used for separating different immature age classes.

RESULTS

Moult of albatrosses

Moult data were obtained from four albatross species. First year juveniles of Atlantic Yellow-nosed and Black-browed Albatrosses do not moult any feathers during their first winter, but Wandering and Tristan Albatrosses, which fledge earlier (at December of the previous year) moult contour feathers (n = 29, all species pooled). One year after fledging (Age 1) all albatrosses moult contour and tail feathers but not wing feathers (n = 14 birds, all species pooled). The first wing (primary) moult and second tail and contour moult occurs during the second year at sea (Age 2) (n = 8 birds). Immature Atlantic Yellow-nosed and Black-browed Albatrosses start wing moult earlier than adults and are found in heavy moult in February, whereas adult breeders moult only contour feathers during the austral summer when they breed (Cuthbert *et al.* 2003b) (n = 16), but start wing and tail moult at the end of the breeding period (~April/May). Three out of 16 breeding Atlantic Yellow-nosed Albatrosses appeared to be failed birds or birds in sabbatical year, which started moult before successful breeders. The same three birds were the only ones to moult rectrices. However, these three plus another five others were moulting contour feathers, suggesting that some body feather moult occurs during breeding. From 26 birds 'Age 2' or over, i.e. moulting or with signs of having had at least one primary moult, nine had two descendant moult waves, one had three waves, and 16 had at least one descending wave. In nine out of 26 birds 'Age 2' or over it was evident that the three outer primaries formed a single series more abraded and clearly of different ages in comparison with inner primaries.

Two out of eight adult Wandering Albatrosses were moulting outer primaries, descendantly, one of which was an adult in sabbatical year and the other of unknown breeding status. All adults had wings with blocks of new and old feathers. Seven adults were moulting tail feathers (scores from 1 to 31), and all were in contour moult, including three ringed birds that at the time of capture were known to be rearing chicks in South Georgia (A. Wood and R.A. Phillips, British Antarctic Survey, *in litt.*).

Thirteen Atlantic Yellow-nosed Albatrosses moulting more than one rectrix were analyzed for tail moult patterns. Most birds were growing four feathers, but up to seven feathers were recorded growing simultaneously. Overall, tail moult score averaged 28.5 ± 21.3, and was very asymmetrical with only two birds having the same summed score for both sides of the tail and just a single bird with all individual feather scores from both sides matching exactly. Moult occurs preferentially in alternate feathers (e.g. scores 020200000202 or 415150554151 or 355151551551 or 001002201010), with a clear pattern of alternating fully grown (old or new) and partially grown feathers. Two Black-browed, two Tristan and six Wandering Albatrosses in active tail moult all had this pattern of alternate tail moult.

Spectacled Petrel moult

All 64 Spectacled Petrels (*Procellaria conspicillata*) sampled from February to August were moulting. The four birds captured in August were in a final moult stage (=contour moult). Primary moult was sequential and descendent, starting at P1 and proceeding to P10 (Fig. 5.2), but at least four birds were in a moult stage that allowed us to be certain that P2 was shed before P1. From one to six inner primaries were shed simultaneously in a row, creating large gaps, as illustrated by the similar score in feathers P1 to P4 (Fig. 5.2). However, a maximum of four outer primaries were growing simultaneously and in different stages. Primary moult scores were high in February and June, probably with our samples composed mainly by immature or failed breeders in February and adults in post-nuptial moult during the second peak in June (Fig. 5.3). The drop in wing moult scores during April was probably because this group included adults and failed breeders started moulting, still with several old feathers (Fig. 5.3). Regarding tail feathers, up to eight feathers were growing simultaneously in alternate pattern (e.g. R1, R3 and R5 or R6, R4 and R2). Tail moult starts at the end of primary moult and by early August all primary and tail feathers were replaced (Fig. 5.3). Fifty-six birds (87.5%) were moulting contour feathers, suggesting a continuous process throughout the moulting period and overlapping with breeding. Apparently, there were no first year (juvenile) birds in our samples.

Great Shearwater moult

Sixty-eight Great Shearwaters were sampled from February to early June, seven of which had no moult throughout the period and so were regarded as first year juveniles. However, we could not exclude the possibility of breeding birds from Tristan da Cunha Islands performing foraging trips to Brazil. Fifteen birds had only contour feathers moulting and could be 'Age 1' birds that skipped wing and tail moult or birds which had moulted earlier. Up to five primary feathers were shed simultaneously (usually 3 or 4), but the block of moulting inner primaries had frequently slightly different ages. Moult was sequential and descendent, starting at P1 and proceeding to P10 (Figs. 5.4 & 5.5), but in at least five birds, P2 had been shed before P1. Outer primaries usually do not shed at the same time, with the two or three outermost feathers usually in growth with slightly different sizes. The pattern of tail feather moult was not clear, but there is a tendency for moult starting at inner tail feathers and proceeding outwards rather than the alternate pattern described for Spectacled Petrels and albatrosses above. Tail moult starts when the first block of inner primaries is fully grown, i.e. half way through the primary moult and not at the end of primary moult as in albatrosses and *Procellaria* Petrels. Fifty-five birds (82%) were moulting contour feathers, which show that these feathers are usually replaced throughout the moulting period (see Fig. 5.1 for a typical individual moulting primary, tail and contour and coverts moult). Just a few Great Shearwaters were observed around the vessel during July to September, and all those ones observed closely had new primaries, tail and coverts.

Moult of other species

Moult of other species sampled in this study is summarized in Table 5.1. All Southern Giant Petrels were first year juveniles (based on plumage color), and so were not moulting. Similarly, all Southern Fulmar, Cape Petrels and 20 out of 30 White-chinned Petrels had no moult by August, and could be either first year juveniles or adults which had already finished moult. One adult Northern Giant Petrel had completed moult by August, similar to adult Black-browed Albatrosses, which suggests that these birds would leave for breeding grounds soon (Hunter 1984b). Remarkably, the number of rectrix pairs in Southern Fulmar was variable, most birds with the typical six pairs, one bird with seven pairs and two with eight pairs.

A single White-chinned Petrel had primaries moulting in late May, with two outermost feathers growing (score 47), plus tail (score 10) and contour moult. Only two other birds were moulting tail feathers (scores 26 and 59) while ten birds were moulting contour feathers. Apparently moult occurs during autumn and is completed by early winter as could be the case in other fulmarine petrels studied here.

DISCUSSION

The current study confirms that albatrosses (Diomedeidae), petrels and shearwaters (Procellariidae) differ in moult patterns and phenology. Albatrosses do not replace all wing feathers in a year, but overlap tail and contour moult with breeding, with descendant moult in several waves resulting in blocks of different aged feathers. Petrels and shearwaters start to moult early in the breeding season and replace all wing and tail feathers every year in a single descendant wave, starting at either P1 or P2. Albatrosses and petrels moult many tail feathers simultaneously, asymmetrically, but alternating full grown with shed or growing feathers, while shearwaters moult tail feathers in an outward wave. Overlap between moulting and breeding is demonstrated to be common with tail and contour feathers, but limited in wing, which suggests that flight constraint rather than nutritional and energetic limitations is the ultimate factor determining primary moult timing.

Regarding moulting of Atlantic Yellow-nosed, Black-browed, Wandering and Tristan Albatrosses, this study confirms that primary moult does not overlap greatly with breeding as is also the pattern for other albatrosses (Furness 1988; Prince & Rodwell 1994; Prince *et al.* 1993; Edwards 2008). However, for Atlantic Yellow-nosed Albatross, moult could starts at the end of breeding, and body and tail moult overlap with breeding at least in some individuals as we also demonstrated for breeding Wandering Albatross.

Primary moult in Atlantic Yellow-nosed Albatross and other albatrosses takes place usually in two waves (sometimes three or four) as described previously from observations on breeding grounds (Furness 1988). Moult takes at least two years to complete in adults, and full primary moult occurs in immatures, which are not constrained by reproduction; moult of the three outer primaries are clearly descendant in adults and immatures, whereas innermost primaries appear to be moulted descendantly in some birds, which agrees with the pattern described for the Waved Albatross (*Phoebastria irrorata*) (Harris 1973), and for immature Gray-headed and Black-browed Albatrosses, but differs from adults of the latter species which have outer descendant and inner ascendant moults (Prince *et al.* 1993). Albatrosses studied here do not carry out any moult during the first year after fledging, but moult tail and body feathers annually starting at Age 1, but only undergo partial wing moult, a pattern also demonstrated for other albatrosses (Prince *et al.* 1993; Langston & Rohwer 1996) and petrels in the current study.

Age 1 Black-browed Albatrosses were captured and differed from first year birds by having moulting rectrices and contour feathers in May, but only moulting contour feathers in August. Primary feathers were all of the same age and birds do not appear to

moult P8-P10 and some inner primaries during the second winter, as suggested previously (Prince *et al.* 1993; Tickell 2000).

Tail moult in albatrosses and petrels was asymmetrical and several feathers could moult simultaneously, even for breeding birds, in agreement with their lesser importance in flight (Bugoni *et al.* 2007). The pattern of tail moult for the four albatrosses and Spectacled Petrel studied in detail shows that feathers are replaced in alternate order rather than in a simple inward wave as suggested by Tickell (2000) for albatrosses, and differs from the outward pattern we recorded in Great Shearwaters. The annual replacement of tail feathers in albatrosses and petrels was confirmed (Prince *et al.* 1993; Tickell 2000).

Primary moulting in petrels was simpler than in albatrosses. Petrels and shearwaters (both Procellariidae) follow a single descendant wave moult starting at inner primaries towards the outer P10 feather. Primary moult also differs from that in albatrosses by being annual and starting during the breeding season, sometimes very early. The pattern of primary moult starting at P2 rather than with P1 in Sooty Shearwater (*Puffinus griseus*) and Northern Fulmar (Thompson *et al.* 2000; Allard *et al.* 2008) and that P1 is functionally similar to secondaries and moults as a single block together with outer secondaries in Laysan and Black-footed Albatrosses (Edwards & Rohwer 2005), was confirmed here for Spectacled Petrel and Great Shearwater. We could anticipate that this pattern could be found to be widespread among Procellariiformes if birds were sampled early in the primary moult. This pattern, which could appear trivial at first glance, has important implications in other studies using feathers, such as for measuring pollutants and stable isotopes. For example, it has been demonstrated that the first primaries and first tail feathers to be moulted have higher concentration of mercury due to accumulation in the body and elimination through feathers. Thus, our results of P2 being replaced first explain the previously unexplained observation that P2 has higher mercury levels than P1 in some southern Atlantic Ocean petrels (Furness *et al.* 1986).

We report here that the number of rectrices in Southern Fulmar is variable, from the typical six pairs (Marchant & Higgins 1990) to the seven pairs also found in Cape Petrel (Beck 1969, this study) or the eight pairs of Giant Petrels (Marchant & Higgins 1990, this study). These four species are all from the fulmarine taxa and could explains the departure from the six pairs of rectrices found in other Procellariidae and birds in general (Ginn & Melville 1983; Marchant & Higgins 1990) due to phylogenetic constraint rather than having a functional role.

We found no moult in Cape Petrels and Southern Fulmars, and reasonably assumed that birds were juveniles, which do not moult during the first winter. Beck (1969) reported

that Cape Petrels delay moult until late in the chick fledging period with tail moult starting at the end of the primary moult and all rectrices shed almost simultaneously. Differently, Barbraud & Chastel (1998) mention that Southern Fulmars starts moult early in the breeding season. Adults of these species and the White-chinned Petrels could have started moult during the breeding and thus finished moult by August when they were sampled in our study.

The breeding season of Great Shearwaters in Tristan da Cunha Islands is from mid-September to mid-May (Cuthbert 2005), a period during which we captured many birds in primary, tail and contour moult. However, Stresemann & Stresemann (1970) and Brown (1988) suggest that Great Shearwaters delay moult until arriving in the northwest Atlantic Ocean and that breeding and moulting do not overlap. This pattern is assumed to be the rule in the literature, despite some records of Great Shearwaters moulting in the South Atlantic Ocean and supposed to be immatures which migrate from the North Atlantic to moult (Watson 1971; Bourne 1995). The abundance and records of birds in Brazilian waters suggested an increase in numbers during the northward migration in austral autumn (Metcalf 1966; Neves *et al.* 2006b) and again an increased abundance late in austral spring (Neves *et al.* 2006b), which could be a southward migration of immatures or an influx of adult birds in the pre-laying exodus (Bourne 1995). However, results presented here demonstrate that the SW Atlantic holds important numbers of moulting Great Shearwaters during summer and early austral autumn. Furthermore, transequatorial migrant Cory's Shearwaters (*Calonectris diomedea*) can start wing moult in the breeding grounds (Monteiro & Furness 1996) and, presumably, complete in winter quarters, so we could speculate that at least some Great Shearwaters may start moulting before the migration to the northern hemisphere. After moulting the innermost block of up to six feathers, other feathers are shed one by one, which may cause only minor effects on flight efficiency and allow migration. It would be interesting to test this hypothesis by checking if Great Shearwaters recently arrived in the North Atlantic Ocean have new inner and old outer primary feathers. Adults and immatures appear to have different moult areas and periods, the former in the northwest Atlantic off Canada and Greenland during the boreal summer, and the latter in the southwestern Atlantic Ocean during the austral summer.

Weimerskirch (1991) reported that some Wandering Albatross breeders start moulting primaries during the last months of chick rearing, while Edwards (2008) mentioned that far less than 1% of albatrosses overlap breeding and moulting. We sampled breeding Atlantic Yellow-nosed Albatrosses (as indicated by presence of a well developed brood patch or enlarged gonads) and it is improbable that our sample contained a large number of failed breeders or immatures with plumage, bill color and moult characteristics of adults. Thus we would suggest that primary moulting starts before the end of breeding in

many individuals of this species. However, we have shown that tail and contour feather moult overlaps greatly with breeding in Atlantic Yellow-nosed and Wandering Albatrosses. This difference between wing and tail/contour moult presumably arises from the key importance of wing feathers in the flight of large distance foragers such as albatrosses (Edwards 2008). Based on the evidence of overlapping breeding and tail/body moult, but seldom wing moult, and also the much more asymmetric moult of tail feathers in comparison with wing (Furness 1988; Prince *et al.* 1993; Bugoni *et al.* 2007), we suggest that impairing wing condition rather than the frequently postulated nutritional limitations (e.g. Hunter 1984a; Weimerskirch 1991; Tickell 2000; Allard *et al.* 2008) is the key factor behind avoiding wing moult during breeding (Tucker 1991; Edwards 2008). Reduced flight efficiency as the ultimate factor explain more satisfactorily why birds undertaking extended foraging trips during breeding, as is the case in albatrosses, do not moult wing feathers during breeding, whereas the similar sized Giant Petrels, which feed on penguin and seal carrion near colonies, start moulting primaries at egg or early chick stages (Hunter 1984a). In a multi-species analysis, Bridge (2006) also concluded that non-migratory Procellariiformes are more likely to overlap breeding and moulting than migratory species. Obviously, energetic constraint could arise as a result of flight impairment, but it does not appear to be the primary limiting factor. It is also strongly suggestive that roughly half of the species of Procellariiformes overlap some sort of moult (of tail and/or contour feathers and sometimes wing feathers) with breeding (Bridge 2006), contrary to the hypotheses of energetic limitations and the assumption that breeding/moulting overlap is an exception (e.g. Prince *et al.* 1993; Cooper *et al.* 1991; Edwards 2008). As previously suggested by Monteiro & Furness (1996) data on tail and body moult shown here, usually not recorded in moult studies, do not agree with the commonly presented hypotheses of nutritional and energetic constraints limiting timing of moult. Patterns of tail and body moult during breeding and in other species such as winter breeders, as well detailed studies investigating whether and how suspended moult occurs in petrels, will probably provide further insights on suggestions presented here. Such studies could also help us to understand patterns of nutrient allocation for moult and breeding in Procellariiformes.

Feather growth rate appears to be physiologically limited in birds, with growth rates of individual feathers varying between 2.5 to 10 mm per day in birds of different orders of magnitude of body size, from small passerines to large albatrosses (Langston & Rohwer 1996). An alternative for large birds to overcome this time limitation could be to grow more feathers simultaneously, which has nutritional and aerodynamic disadvantages (Langston & Rohwer 1996). Being constrained by the time available for moult and avoiding excessive overlap of energetically demanding activities as breeding, migration and moulting, Procellariiformes studied here seem to adopt two distinct strategies: petrels start to replace feathers during the breeding period and replace more primaries

and tail feathers at one time, and have annual moult, whereas albatrosses undergo long moulting cycles, replacing less feathers at once and suspending the primary moult during breeding periods to avoid impacts on flight efficiency, but still carrying out moult of tail and contour feathers at the same time as reproduction.

Table 5.1. Percentage of petrels and shearwaters in wing (primary), tail and contour feather moult captured at sea during summer/autumn (February to early June) and winter (late July to early September). Average scores of primary and tail moult is given in parentheses. -- Not sampled.

Species	Summer/Autumn				Winter			
	Wing	Tail	Contour	n	Wing	Tail	Contour	n
Southern Giant Petrel	--	--	--	--	0	0	0	11
Northern Giant Petrel	--	--	--	--	0	0	0	1
Southern Fulmar	--	--	--	--	0	0	0	10
Cape Petrel	0	0	0	1	0	0	0	31
White-chinned Petrel	8.3(47)	16.6(18)	25	12	0	5.6(59)	38.9	18
Sooty Shearwater	--	--	--	0	50(49)	50(4)	50	2
Cory's Shearwater	100(48)	2(60)	100	2	--	--	--	--
Wilson's Storm-petrel	50(50)	50(50)	50	2	0	0	0	2

Figure 5.1. Great Shearwater (*Puffinus gravis*) moulting plumage in southern Brazil. Wing feathers (primary remiges) are numbered P10 (outer) to P1 (inner). Note that P9 and P10 in both wings are brownish, paler and with ragged tips characteristic of old feathers, while P1 to P8 are new feathers blackish, bright and with rounded tips. Tail feathers (rectrices) are numbered R6 (outer) to R1 (inner). R1 and R5-R6 are recently grown feathers, whereas R2 to R4 are old. Note also differences between old (brown) and new (black) feathers on wing coverts and dorsum.

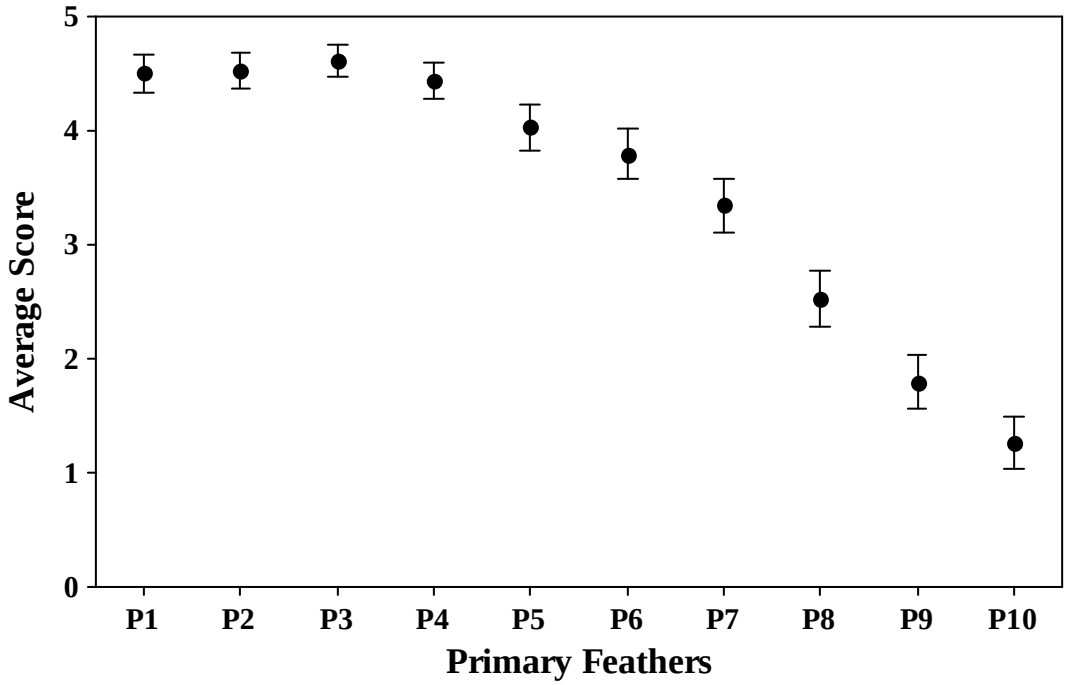

Figure 5.2. Primary moult score of individual feathers (average ± 1 SD) of Spectacled Petrels (*Procellaria conspicillata*) captured at sea in the southwestern Atlantic Ocean (n = 64 birds).

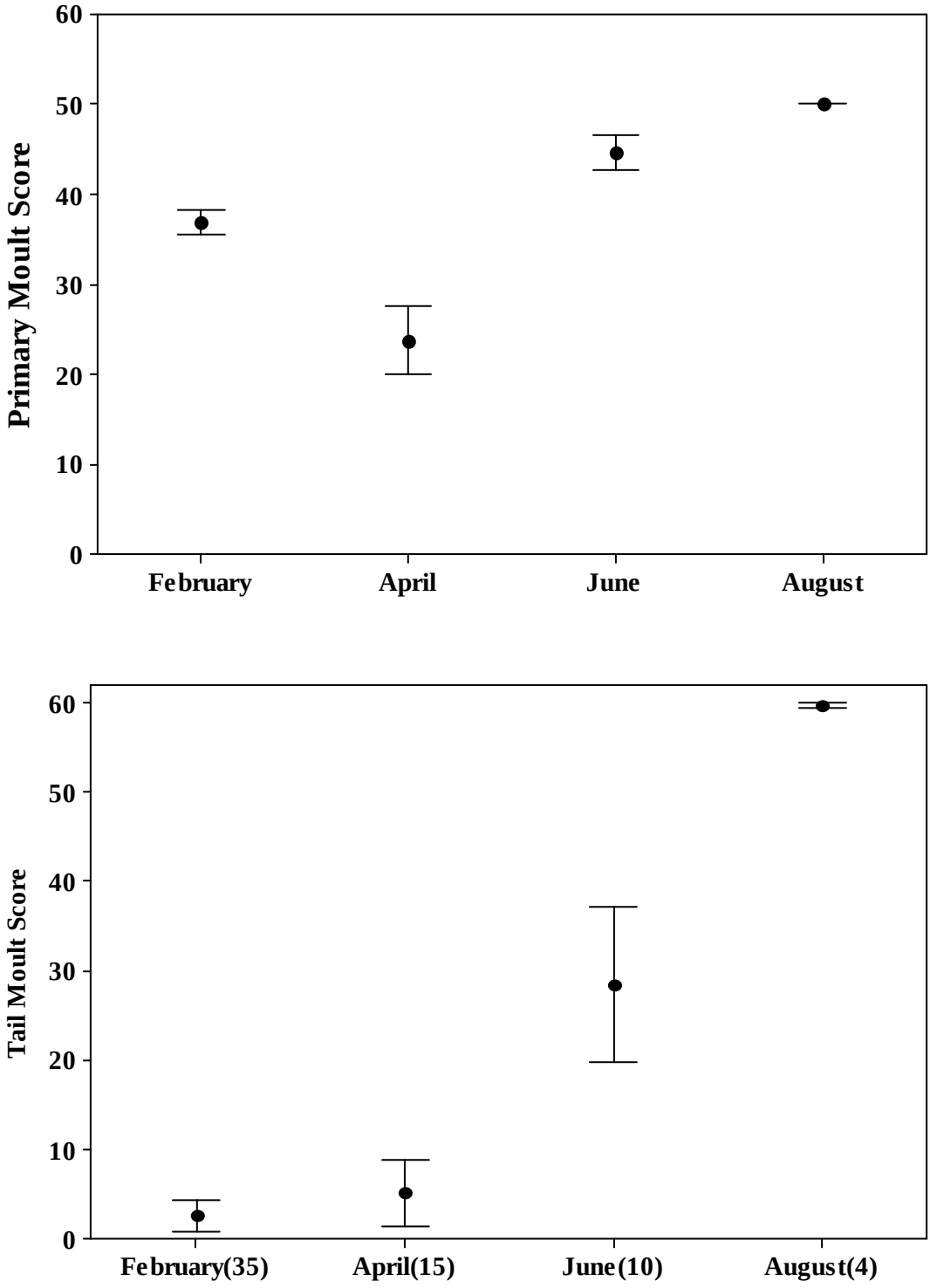

Figure 5.3. Moult scores of wing primary feathers (above) and rectrix feathers (below) of Spectacled Petrels (*Procellaria conspicillata*) captured at sea in the southwestern Atlantic Ocean in different months. Values are given as average ± 1 SD, and sample size in parenthesis for each month.

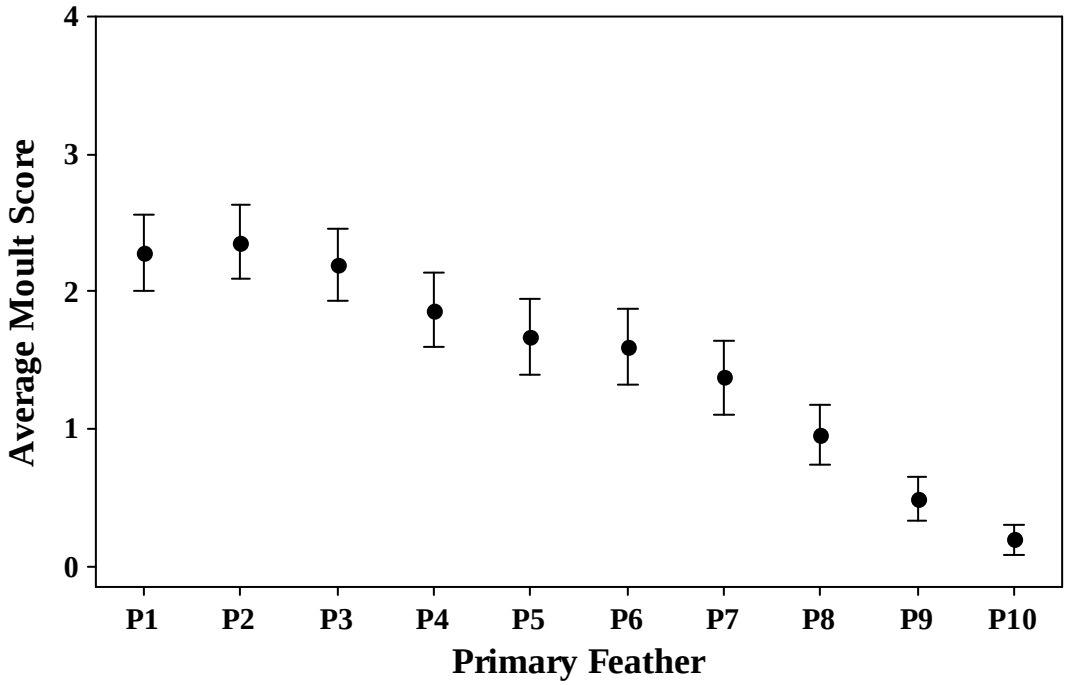

Figure 5.4. Primary moult score of individual feathers (average ± 1 SD) of Great Shearwaters (*Puffinus gravis*) captured at sea in the southwestern Atlantic Ocean (n = 68 birds).

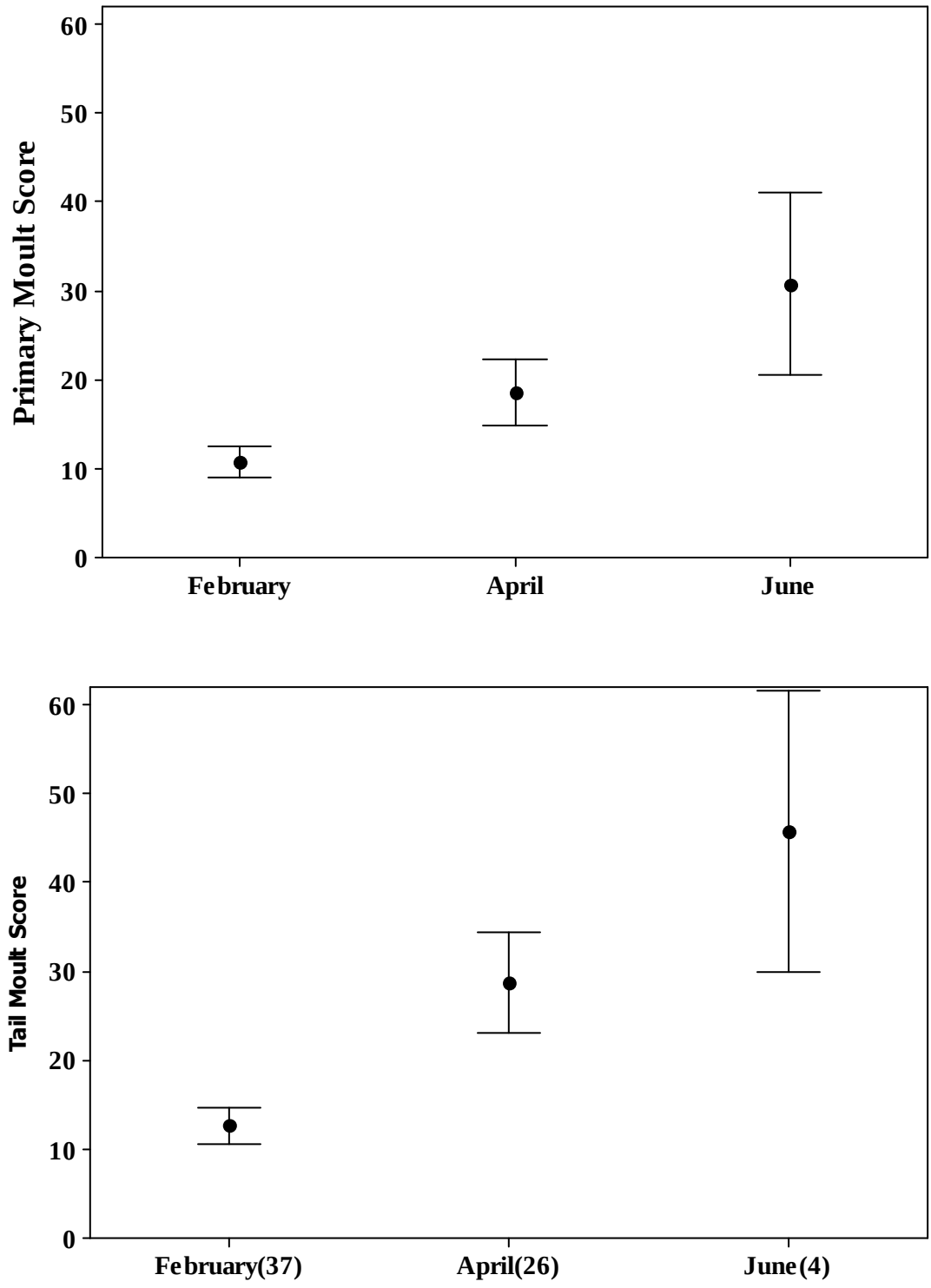

Figure 5.5. Moult scores of wing primary feathers (above) and rectrix feathers (below) of Great Shearwaters (*Puffinus gravis*) captured at sea in the southwestern Atlantic Ocean in different months. Values are given as average ± 1 SD, and sample size in parenthesis for each month.

Chapter 6

SEXUAL SIZE DIMORPHISM AND AGE COMPOSITION OF ALBATROSSES AND PETRELS OFF BRAZIL

Bugoni L, Furness RW. *S*exual size dimorphism and age composition of albatrosses and petrels off Brazil. **Under review**

ABSTRACT

We present data on sexual size dimorphism (SSD) and age composition of albatrosses, petrels and shearwaters in southern Brazil for 301 birds of 14 species captured live at sea. SSD was more pronounced in bill measurements than in other traits, and more conspicuous in Giant Petrels and *Diomedea* albatrosses. Closely related species pairs of *Thalassarche* albatrosses and *Procellaria* petrels had differing levels of sexual dimorphism. All linear external traits measured within *Thalassarche* and *Procellaria* overlapped considerably and despite being significantly different were not adequate for species determination or sexing. The pelagic community of seabirds off Brazil comprises birds from different ages and breeding status according to species. While juveniles of some species, such as Black-browed Albatross (*Thalassarche melanophris*) and Southern Giant Petrel (*Macronectes giganteus*) predominated, all age-classes (including breeding birds) were recorded for Atlantic Yellow-nosed (*T. chlororhynchos*) and Wandering (*Diomedea exulans*) Albatrosses.

INTRODUCTION

Information on some Brazilian seabird species has been obtained mostly or exclusively through beached carcasses (Olmos *et al.* 1995; Martuscelli *et al.* 1997; Sick 1997; Bugoni *et al.* 2003; Lima *et al.* 2004; Bugoni 2006) or studies focused on incidental capture in fisheries (e.g. Neves & Olmos 1997; Olmos *et al.* 2001; Neves *et al.* 2007; Bugoni *et al.* 2008a, c). Few studies have described the pelagic community at sea through census of birds attending bottom longline vessels (Olmos 1997; Olmos & Bugoni 2006), or through snapshot and continuous censuses (Neves *et al.* 2006b). Overall, ten albatross (Diomedeidae) and 31 petrel (Procellariidae, Hydrobatidae and Pelecanoididae) species have been recorded in Brazil (CBRO 2007), with only two species breeding in Brazilian territory (the Trindade Petrel *Pterodroma arminjoniana*, and the Audubon's Shearwater *Puffinus lherminieri*). The pelagic seabird community in Brazilian waters is generally thought to be composed by (1) birds which breed elsewhere and migrate to the area during the non-breeding period, (2) birds that reach the area during breeding periods when they perform long foraging trips, (3) first year juveniles and older but still immature birds (Neves *et al.* 2006b).

The recent development of a non-destructive method for trapping seabirds at sea from fishing vessels (Bugoni *et al.* 2008b) allowed us to sample a range of healthy albatross and petrel species attending vessels. Here we describe the community in terms of age composition, and determine whether linear measurements can be used to distinguish similar species frequently captured in fisheries. We extend knowledge of the age classes of birds present off Brazil, and investigate sexual size dimorphism of birds sexed by molecular methods.

MATERIAL AND METHODS

Study area and seabird trapping

Albatrosses and petrels were captured as described by Bugoni *et al.* (2008b, Chapter 2), from vessels using a range of hook-and-line and pelagic longline fishing methods targeting Tuna (*Thunnus* spp.), Sharks (mainly Blue Shark *Prionace glauca*) and Swordfish (*Xiphias gladius*). This fleet operates in deep waters over the continental shelf and offshore waters in southern Brazil throughout the year (Mayer & Andrade 2005) in the region under the influence of the Subtropical Convergence, formed by the meeting of the warm tropical Brazil Current flowing southward and the cold Falkland (Malvinas) Current flowing northward (Garcia 1998). The overlap between fishing fleet and distribution of albatrosses and petrels is cause for concern due to incidental bycatch of these seabirds on longline hooks, mainly from June to November (Bugoni *et al.* 2008a, Chapter 9). Birds were attracted close to the vessel using baits and shark liver and captured using a cast net (Bugoni *et al.* 2008b, Chapter 2). Captures occurred during six cruises and 58 trapping days from February to June 2006 and July to September 2007, from 25 to 35ºS. In addition, 24 birds incidentally captured in hook and line fisheries taking place onboard were sampled from February to May, but not in August-September.

Measurements

Measurements of birds were taken using vernier calipers with accuracy of ± 0.1 mm and included exposed culmen (=bill) length, from the bill tip to the point where feathers begin to hide the culmen; bill depth at the base of the bill; and tarsus (=tarsometatarsus) length, measured from the middle tarsal joint to distal end of tarsometatarsus (with foot closed towards tail). Wing chord length, from the carpal joint to the tip of the longest primary feather of the right wing, was taken using a stopped rule; and tail length obtained with a metal ruler, from the point where the longest tail feather emerged from skin, by inserting the rule parallel to tail feathers. Both wing and tail were measured with accuracy of ± 1 mm and were not taken from birds moulting the outer primaries or longest tail feathers, respectively. All measurements were carried out by the same researcher throughout the period.

Wandering (*Diomedea exulans*) and Tristan (*D. dabbenena*) Albatrosses are virtually identical in plumage (Onley & Scofield 2007), so we used the discriminant function in Cuthbert *et al.* (2003a) to assign species. We further confirmed identification, after sexing birds by molecular methods, using culmen length (see Cuthbert *et al.* 2003a for details).

Ageing

Pictures of bill and general plumage were taken from albatrosses and Giant Petrels for ageing. Giant Petrels change plumage colour from wholly dark brown to pale brown-

greyish with age, which was also used to identify juveniles from older birds. Black-browed Albatrosses (*Thalassarche melanophris*) change bill colour with age, which combined with wing moult allows age determination up to six years-old (Prince & Rodwell 1994). The same patterns of bill colour changes and moult were described for Grey-headed Albatrosses (*T. chrysostoma*) (Prince & Rodwell 1994). Ageing of Atlantic Yellow-nosed Albatrosses (*T. chlororhynchos*), which change from a wholly dark bill to orange at the culminicorn, ramicorn and ungues (Chapter 3) was used for ageing this species. Birds not moulting during or after the breeding season were considered first year juveniles.

Molecular sexing

Blood samples (1 mL) were taken by syringe and needle from the tarsal vein of every bird. Subsamples of blood were stored in 1.5 mL vials preserved with absolute ethanol. Sexing of birds was carried out after DNA extraction and PCR amplification of CHD genes using primers 2550F (Fridolfsson & Ellegren 1999) and 2757R (R. Griffiths, umpublished) and genes separated in 2% agarose gel by electrophoresis. Briefly, copies of CHD genes are present in both Z and W, the bird's sex chromosomes. CHD-Z and CHD-W genes differ in the base pair length of their non-coding regions. Because females are heterogametic (ZW) and males homogametic (ZZ), the separation of gene amplification products by size results in a single band for males and two bands for females. DNA extraction negative controls were included for every 23 samples. Positive and negative controls were included for each PCR reaction and 1/3 of all DNA extractions were repeated to confirm gender assignment. Furthermore, we used samples of previously sexed birds as controls: one male Atlantic Yellow-nosed Albatross killed in fisheries and sex determined by necropsy, and four ringed Wandering Albatrosses which we sexed but subsequently discovered were of known sex from observations at the breeding colony (A. Wood and R.A. Phillips, British Antarctic Survey, *in litt.*). All these birds were correctly sexed by the molecular method.

Data analysis

Distribution of biometric data was inspected graphically to detect outliers and odd values, which were rechecked against field datasheets and corrected. Unrealistic values were removed, but some apparent outliers were from consistently small or large individuals, thus were maintained. Measurements are presented as average $\pm$ 1 standard deviation and range (minimum and maximum) for males and females.

Sexual size dimorphism was calculated for all variables as the ratio between average values of females and males. Statistical differences were tested by the non-parametric Mann-Whitney test (Fowler *et al.* 1998), which overcomes problems associated with non-normal distribution and heteroscedasticity. Furthermore, we considered that reversed sexual dimorphism (i.e. females larger than males) could occur as it is frequent in

seabirds (Catry *et al.* 1999; Bridge 2006; Onley & Scofield 2007) and so we assumed the same probability of males being larger or smaller than females by conducting two-tailed tests (Fowler *et al.* 1998). Comparison between biometric values of closely related pairs of species was carried out to provide support for identification of birds incidentally killed in fisheries or partial specimens from stranded or fishery killed birds. For this analysis Mann-Whitney test were used and Bonferroni correction for multiple comparions applied, thus the threshold P-value become 0.01 (the new P-value is derived from the ratio between the previous P-value of 0.05 and the number of traits compared, i.e. 5: wing, tarsus, culmen and tail lengths, and bill depth). Statistical tests were carried out using BioEstat software version 5.0 (Ayres *et al.* 2007) and Minitab® Software v. 15.1 (Minitab Inc., Ltd.).

RESULTS

In total, 301 birds from 14 species were captured, including nine petrels and shearwaters (Procellariidae), four albatrosses (Diomedeidae) and one storm-petrel (Procellariidae) (Table 6.1). All birds from the 14 species studied were successfully sexed using primers 2550F and 2757R.

Sexual size dimorphism

Males were larger than females in all species studied, although there was considerable overlap between sexes in linear measurements (Table 6.1). SSD was more conspicuous for Southern Giant Petrel (*Macronectes giganteus*) where females were 82% of the size of males, and for Wandering (*Diomedea exulans*) and Tristan (*Diomedea dabbenena*) Albatrosses (Table 6.2). SSD was less pronounced or absent for some traits in Great Shearwater (*Puffinus gravis*).

Pairs of closely related species, as for example *Thalassarche* albatrosses or *Procellaria* petrels, did not show consistent sexual dimorphism in size (Table 6.2). SSD is more accentuated for Atlantic Yellow-nosed Albatross than for Black-browed Albatross and for Spectacled Petrel (*Procellaria conspicillata*) than for White-chinned Petrel (*Procellaria aequinoctialis*).

Morphometric differences in sister species and in relation to age

Atlantic Yellow-nosed Albatross males and females are generally smaller than Black-browed Albatross (Table 6.1). For males, significant differences after Bonferroni correction were detected only for wing length (Mann-Whitney test $U = 3.6$, $P = 0.0003$), while for females significantly different traits were culmen ($U = 2.6$, $P = 0.009$), bill heigth ($U = 4.1$, $P < 0.0001$), tarsus ($U = 4.5$, $P < 0.0001$) and wing ($U = 3.9$, $P < 0.0001$); tail length was higher in female Atlantic Yellow-nosed Albatrosses, but non-

significant (U = 1.8, P = 0.07). However, measurements overlapped greatly and species separation based solely on measurements is not feasible.

Spectacled Petrel was slightly smaller than White-chinned Petrels for all parameters (Table 6.1), except for bill depth, but values were not significantly different for most morphometric measurements (culmen, bill depth and tarsus) for both males and females after Bonferroni correction (P < 0.01). The only significant differences were for non-skeletal traits, i.e. wing (U = 2.9, P = 0.004 for males; U = 2.9, P = 0.004 for females) and tail length (U = 5.8 P < 0.0001 for males; U = 3.4, P = 0.0006 for females). In addition, all distributions of measurements overlapped considerably, precluding separation of individual birds to species even when the sex of the bird was known.

For Great Shearwater we tested for differences in size arising with age by comparing juveniles and adults. No difference was found for tarsus, wing, and tail (Mann-Whitney test, all P > 0.05, males and females pooled as SSD was not detected, see above).

Age composition of the community

The community of Procellariiformes in the SW Atlantic was composed mostly of juveniles of some species such as Black-browed Albatrosses, Southern Giant Petrels, and probably Cape Petrels (*Daption capense*) and Southern Fulmars (*Fulmarus glacialoides*), but for other species, a mix of immatures and adults, such as White-chinned Petrel, Great Shearwater and the Atlantic Yellow-nosed Albatross was found (Table 6.3). For a few species the adults include breeders that forage in the region during the breeding period (e.g. Wandering and Atlantic Yellow-nosed Albatrosses), as indicated by moult condition, presence of a brood patch or rings identifying Wandering Abatrosses as individuals known from studies at the breeding colony to have chicks at the time of their capture off Brazil.

DISCUSSION

The range of measurements of body traits for each species presented in this study was variable (see SD in Table 1), probably a result of birds from different origins that meet in the area, as size of species may vary considerably according to population. For the Southern Giant Petrel, for example, Copello *et al.* (2006) provided linear measurements from Argentina and reviewed values from other Antarctic and sub-Antarctic locations, and demonstrated that birds from Patagonia, Gough and Falkland (Malvinas) Islands are smaller than in other populations. Measurements for males and females we captured at sea cover the wide range of values of different populations and are an indication of different origins of sampled birds, in line with records of banded birds from known locations and diagnostic plumage characteristics (Olmos 2002; Carlos *et al.* 2005). For instance, we sampled one Southern Giant Petrel male consistently smaller than others, which probably belonged to the *M. giganteus solanderi* taxon. Multi-origin is also

possible, as demonstrated for the Black-browed Albatross (Phillips *et al.* 2005b; Chapter 3) and for other species (see review of ringed seabirds recovered in Brazil in Olmos 2002).

Hall (1987) and Ryan (1998) showed that the Spectacled Petrel was smaller in most morphometric measurements (except tarsus and minimum bill depth) in comparison with White-chinned Petrels, which, among other characteristics, warrant it as a biologically valid species (Ryan 1998). Here we confirm that both male and female Spectacled Petrels are on average slightly smaller than White-chinned Petrels. However, in our sample of both species captured at sea, interspecific differences between linear measurements of skeletal traits (culmen, bill depth at base and tarsus lengths) were not detected. On the other hand, wing and tail length were highly significant different, which confirms suggestion of Rowan *et al.* (1951). These results contrast with Ryan (1998) who found differences in 20 Spectacled Petrels measured on the breeding grounds in Inaccessible Island, Tristan da Cunha Group, compared with 72 White-chinned Petrels incidentally captured in longline fishery around Prince Edward Island, Indian Ocean. He found differences for culmen, bill depth at base, bill depth at nail, wing and mass, but not in bill minimum depth and tarsus. Morphometric differences, segregation in breeding islands, differences in vocalizations and different breeding periods were used to assure full specific status for the Spectacled Petrel (Ryan 1998). Results of measurements presented here from a pool of birds at sea, and controlling for sex were slightly different, but based on other evidences provided by Ryan (1998) and clear at sea segregation between Spectacled and White-chinned Petrels (Neves *et al.* 2006b) and derived from satellite tracked birds (Chapter 8) confirm the distinctiveness of these taxa.

Sexual size dimorphism is male biased in most avian species and families (Székely *et al.* 2007). In a multi-species analysis Bull *et al.* (2005) noted that *Puffinus* exhibit low levels of SSD, with some species showing reversed dimorphism in some traits, as in wing length of Great Shearwater. We found similar values for wing length in both sexes and reversed SSD in tail length, in agreement with Bull *et al.* (2005). In general, bill depth was the trait with larger sexual dimorphism towards males in *Puffinus* (Bull *et al.* 2005). Our analysis, including more distantly related species such as petrels and albatrosses, found that bill depth and culmen length are the measurements with most pronounced dimorphism, also suggesting the bill as the skeletal feature most prone to be selected for dimorphism in Procellariiformes. SSD in one trait is often only loosely related to SSD in another trait in Procellariiformes, as demonstrated in a range of other bird taxa, suggesting that different selective forces are acting on different traits (Székely *et al.* 2007). Because for several species sampled in this study, birds of distinct populations could have been captured, SSD could be even more pronounced for some of them. Newton (1979 in Hunter 1987) showed that there is a relationship between the type of

prey taken and magnitude of sexual size dimorphism. For Giant Petrels it is suggested that the marked sexual dimorphism in all variables including bill, is related to feeding strategies, with males relying more on terrestrial food (carrion) and females on marine food (Hunter 1987; González-Solís *et al.* 2008). Sexual segregation in foraging areas has been demonstrated for some species with more marked dimorphism in size (e.g. Shaffer *et al.* 2001; González-Solís *et al.* 2008), and could explain the skewed sex ratios in several species (Table 6.2) based on sexual size dimorphism, while niche segregation by diving or feeding tactics is more difficult to demonstrate for sympatric males and females at sea. For other species it is less clear how and why sexual dimorphism evolved, but larger males in all Procellariiformes studied here and more pronounced in bill traits is suggestive that defence of the nest could play a role (Bull *et al.* 2005), but hard to distinguish from sexual selection, which is also a plausible explanation.

Fairbairn & Shine (1993) found that larger seabird species tended to show larger differences in SSD, in their case measured as body mass. Despite problems associated with their analysis as body mass is very variable (see Croxall 1995; Shine & Fairbairn 1995), we found the same pattern using linear single-dimensional measurements, for both albatrosses (Diomedeidae) and petrels/shearwaters (Procellariidae). Weidinger & van Franeker (1998) mention that Cape Petrel is one of the least dimorphic among fulmarines, in agreement with our data (Table 6.2). However, SSD is of the same magnitude or even less conspicuous in some other Procellariiformes studied here, such as White-chinned Petrel, Great Shearwater and Black-browed Albatross.

We also failed to distinguish both sister *Procellaria* and *Thalassarche* species based on linear measurements alone, despite significant differences between some traits. The degree of overlap is high even when the sex is known, and precludes species identification of partial carcasses sometimes collected onboard vessels and frequently found stranded on beaches. Future studies using discriminant function analysis could be more successful in separating these closely related species. The limited differences between sister species could also be due to biases toward some age classes of different species. For example most White-chinned Petrels were juveniles and Spectacled Petrels were adults. Absence of SSD in Black-browed Albatrosses could be because most were juveniles not fully grown.

Age composition of the pelagic community

For some species, most individuals in the area were juveniles, such as with Black-browed Albatrosses, White-chinned Petrels and Southern Giant Petrels. For others, juveniles, immatures and breeding or non-breeding adults shared the area. Particularly remarkable is the presence of a large number of breeding adult Atlantic Yellow-nosed Albatrosses, which breed only in the Tristan da Cunha Islands. It is not clear if breeding Spectacled

Petrels, which also nest on Tristan da Cunha Islands, also forage in Brazilian waters during their breeding period. However, all birds of that species we captured during the breeding period were immatures or adults, with no juvenile, which is surprisingly and suggests that juveniles occur in a different area. Great Shearwater juveniles and immatures occur in the area during the breeding period, with the latter carrying out extensive moult in Brazilian waters. Species from the Antarctic, such as Cape Petrel and Southern Fulmar, occur in the area exclusively during winter-spring and are probably composed mostly by juveniles, but the rapid moult undertaken by these species (Beck 1969; Barbraud & Chastel 1998) precludes accurate age determination of birds we captured in mid-winter. For Wandering Albatrosses, ringed male and female birds rearing chicks in South Georgia Island, as well as juveniles and birds in sabbatical year were captured, demonstrating the importance of the area for all age classes and sexes. This is probably true for Tristan Albatrosses, as juveniles and adults were captured, however none of them had been previously ringed.

Overall, the pelagic community of seabirds off the Brazilian coast is composed of birds from different ages and breeding status according to species. Birds originate from a wide range of breeding areas, including Tristan Islands, Falklands (Malvinas) and South Georgia in the South Atlantic, and Macaronesian Islands in the North Atlantic. Variation in age composition throughout the year could not be addressed here but deserves further study, particularly for species of conservation concern and known to be incidentally captured in fisheries throughout the year in the area (Neves *et al.* 2007; Bugoni *et al.* 2008a).

Table 6.1. Measurements (average ± 1SD, in mm) of wintering albatrosses and petrels of Brazil sampled at sea in 2006 and 2007. For sample size up to 3, raw values are given. The order of values is presented according to each individual. For wing or tail length, measurements could not be taken in some cases due to moult. When this occurred, sample size is provided in brackets.

Species	Sex (n)	Culmen	Bill Depth	Tarsus	Wing	Tail
DIOMEDEIDAE						
Tristan Albatross *Diomedea dabbenena*	Male (3)	147; 148; 157	58.6; 60.3; 63.6	112.7; 120.1; 113.9	(0)	206; 191; 201
	Female (1)	141.7	57.4	111.8	632	184
Wandering Albatross *Diomedea exulans*	Male (5)	169.6±1.7; 168-172	65.6±3.1; 62.3-70.6	124.4±1.7; 122.2-126.1	688.3±13.6; 672-703 (4)	202.8±11.9; 191-216
	Female (4)	161.8±4.6; 157-167	62.8±2.2; 60-64.6	115.4±6.0; 107.7-120.3	647±13.6; 627-656	202.3±8.3; 192-212
Black-browed Albatross *Thalassarche melanophris*	Male (6)	117.2±5.6; 109.6-125.7	45.0±1.9; 43.1-48.1	86.1±3.0; 82.2-89.9	543.5±9.8; 530-560	197.3±12.8; 179-215
	Female (27)	116.6±3.7; 108.3-124	43.7±1.4; 39.9-45.9	83.6±2.2; 79.8-88.4	525.3±12.3; 506-548	184.1±8.3; 170-205
Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*	Male (21)	118.7±4.0; 111.9-128	43.4±1.3; 41.0-45.7	83.1±2.8; 77.3-87.1	500.4±15.4; 465-520 (18)	195.9±7.3; 186-211 (20)
	Female (12)	113.6±2.0; 110.7-117.5 (11)	40.9±1.0; 39.4-42.7 (11)	78.8±2.0; 74.7-81.7	490.5±13.3; 475-515 (8)	189.2±8.1; 175-201
PROCELLARIIDAE						
Fulmariinae						
Southern Giant Petrel *Macronectes giganteus*	Male (7)	95.5±3.3; 92.1-99.8	38.6±1.6; 35.1-39.6	95.0±4.1; 88.0-101.3	525.7±8.6; 513-541	177.4±4.1; 170-182
	Female (4)	82.9±1.1; 81.8-84.4	34±0.4; 33.6-34.5	84.6±2.6; 81.9-88.1	482.8±16.4; 464-497	168.3±8.1; 160-177

Table 6.1. Continued

Northern Giant Petrel *Macronectes halli*	Male (1)	104.7	42	104.1	538	171
Southern Fulmar *Fulmarus glacialoides*	Male (2)	42.8; 45.9	17.7; 15.9	52.4; 54.5	343; 347	124; 119
	Female (8)	41.8±1.8; 39.1-43.7	15.5±0.5; 14.9-16.2	49.8±2.2; 46.7-54.2	327±5.5; 319-335	114.1±4.2; 106-120
Cape Petrel *Daption capense*	Male (22)	31.1±1.1; 29.3-33.3	13.0±0.4; 12.1-13.8	46.3±1.8; 42.6-50.3	271.6±5.2; 260-278	99.2±3.4; 93-104
	Female (9)	30.1±0.7; 29.2-31.2	12.7±0.8; 11.6-14.2	44.5±1.8; 42.0-46.6	266.8±7.6; 257-278	97.4±3.8; 93-104
Procellariinae						
White-chinned Petrel *Procellaria aequinoctialis*	Male (20)	51.8±1.5; 47.5-53.8	22.1±0.7; 21.0-23.2	67.3±2.1; 63.7-70.2	393.7±7.1; 380-408	127.7±4.7; 117-135
	Female (10)	50.0±1.3; 48.4-51.7	20.6±0.7; 19.5-21.8	66.5±2.5; 63.5-71.6	389±12.4; 372-413 (9)	126±5.9; 119-137
Spectacled petrel *Procellaria conspicillata*	Male (46)	52.0±1.5(47.4-55.5)	22.1±0.8(20.8-24.7)	66.6±2.4(62.0-71.0)	374±8.0(355-392, n=35)	116.4±4.3 105-125, n=45)
	Female (18)	49.0±1.8; 46.0-53.9	20.9±0.6; 19.7-21.9	64.6±1.9; 60.9-67.0	363.2±8.0; 355-376 (6)	115.7±4.4; 109-123 (16)
Cory's Shearwater *Calonectris diomedea*	Male (1)	57.4	19.3	58.5	370	134
	Female (1)	52.7	19.4	56.6	-	138
Great Shearwater *Puffinus gravis*	Male (29)	47.7±1.8; 44.5-51.3	16.1±0.7; 14.9-17.3	59.8±2.0; 54.9-64.7	324.9±7.6; 305-340 (24)	111.8±5.6; 100-124 (25)
	Female (38)	46.5±2.0; 41.2-50.1	15.4±0.7; 13.6-16.5	59.0±1.6; 56.2-62.0 (37)	325.4±9.7; 305-345 (32)	113.9±5.5; 98-122 (36)

Table 6.1. Continued						
Sooty Shearwater *Puffinus*	Male (1)	44.4	14.7	55.9	289	77
griseus	Female (1)	42	14.4	59.6	310	92
HYDROBATIDAE						
Wilson's Storm-petrel	1 M, 1 F, 2	12.2±0.5; 11.6-	5.8±0.3; 5.4-	35.5±1.1;	114.3±8.6;	67.5±2.7; 65-
Oceanites oceanicus	unknown	12.9	6.0, (3)	33.9-36.5	133-151	71

Table 6.2. Sexual size dimorphism (female:male ratios) in external body measurements of albatrosses and petrels sampled at sea off Brazil, based on samples reported in Table 6.1. SSD was compared by Mann-Whitney test. NA – not applicable or not available; *ns* – differences not statistically significant; differences significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Species	Culmen	Bill Depth	Tarsus	Wing	Tail
Tristan Albatross	0.94	0.94	0.97	NA	0.92
Wandering Albatross	0.82^{*}	0.96^{ns}	0.93^{*}	0.94^{*}	1.00^{ns}
Black-browed Albatross	0.99^{ns}	0.97^{ns}	0.97^{ns}	0.97^{**}	0.93^{*}
Atlantic Yellow-nosed Albatross	0.96^{***}	0.94^{***}	0.95^{***}	0.98^{ns}	0.97^{*}
Southern Giant Petrel	0.87^{**}	0.88^{**}	0.89^{*}	0.92^{**}	0.95^{ns}
Southern Fulmar	0.94	0.92	0.93	0.95	0.94
Cape Petrel	0.97^{*}	0.98^{ns}	0.96^{*}	0.98^{ns}	0.98^{ns}
White-chinned Petrel	0.97^{***}	0.93^{***}	0.99^{ns}	0.99^{ns}	0.99^{ns}
Spectacled Petrel	0.94^{***}	0.95^{***}	0.97^{***}	0.97^{**}	0.99^{ns}
Great Shearwater	0.97^{ns}	0.96^{**}	0.99^{ns}	1.00^{ns}	1.02^{ns}

Table 6.3. Age classes of albatrosses and petrels samples at sea in 2006 and 2007 in the southwestern Atlantic Ocean. ? = Age status not confirmed.

Species	First year	Immature	Adults	No. of birds sampled
Tristan Albatross	0	1	3	4
Wandering Albatross	1	0	8	9
Black-browed Albatross	25	6	2	33
Atlantic Yellow-nosed Albatross	0	16	17	33
Southern Giant Petrel	11	0	0	11
Northern Giant Petrel	0	0	1	1
Southern Fulmar	10	0	0	10
Cape Petrel	32?	0	0	32
White-chinned Petrel	20?	Present	Present	30
Spectacled Petrel	0	Present	Present	64
Cory's Shearwater	0	1?	1?	2
Great Shearwater	7	15+	45?	67
Sooty Shearwater	0	1	1	2
Wilson's Storm-petrel	3	1	0	4

Chapter 7

THE ROLE OF FISHING DISCARDS ON THE TROPHIC STRUCTURE OF ALBATROSSES AND PETRELS WINTERING IN THE SOUTHWESTERN ATLANTIC OCEAN DETERMINED BY STABLE ISOTOPES

Bugoni L, McGill RAR, Furness RW. The role of fishing discards on the trophic structure of albatrosses and petrels wintering in the southwestern Atlantic Ocean determined by stable isotopes. **Under review**

ABSTRACT

In this study carbon and nitrogen stable isotopes were used to investigate the importance of discards from the tuna-shark pelagic longline fishery in diets of the offshore wintering community of Procellariiformes in the southwestern Atlantic Ocean. It is demonstrated that availability of discard, mainly the preferred shark liver, shared among species with contrasting body masses and feeding techniques, is the cause of overlap and similarity in trophic levels. Carbon isotopic values showed less variation than expected among all the species, and were typical of the subtropical offshore region. Early migrant individuals from Antarctica and sub-Antarctic (e.g. Cape Petrel *Daption capense*, White-chinned Petrel *Procellaria aequinoctialis*, Wilson's Storm-petrel *Oceanites oceanicus*) and species still rearing chicks when sampled (Wandering Albatross *Diomedea exulans*) had carbon isotopic values indicative of austral regions. All southern latitude species underwent a clear shift, in carbon and especially nitrogen isotopes, increasing in trophic level indicating a change in diet between breeding and wintering seasons. Cory's Shearwater *Calonectris diomedea*, the only species not attending vessels, had low nitrogen values reflecting a diet of flyingfish and squid which occurs naturally in the area. While some abundant and widespread petrels and shearwaters, particularly those small in size, benefit from pelagic longline discards, the balance between benefits and the incidental mortality caused by fishing hooks is apparently negative for albatrosses and some petrels due to high bycatch mortality.

INTRODUCTION

Globally, about 7.3 million tonnes of discards from marine fisheries are released every year (Kelleher 2005). Discards from fisheries in the form of undersized fish, commercially unattractive species or offal have been recognized as a key food source for many scavenging seabird species around the world. Fishing activities could have a beneficial effect on seabirds, by bringing them otherwise unavailable food in the form of offal or discarded demersal fish and by removing competitors, or could have direct adverse effects when fisheries deplete stocks of prey of seabirds or drown them in nets or on longline hooks (Montevecchi 2002; Furness 2003). However, the importance and degree of benefits of fishing discards for seabirds is usually difficult to assess and is traditionally inferred from direct observations of birds attending fishing vessels or dietary studies in colonies (e.g. Hudson & Furness 1988; Thompson & Riddy 1995). Around Falkland (Malvinas) Islands, despite thousands of Black-browed Albatrosses (*Thalassarche melanophris*) attending finfish trawlers (Thompson & Riddy 1995; Sullivan *et al.* 2006), this fishery provides only 4.4% of the estimated annual energy requirements of the population. Medium to long-term, or from a community perspective, fishing discards have recently been demonstrated to be detrimental for some species, for example skuas increasing predation upon other seabirds when discard levels decline (Votier *et al.* 2004) or by providing low-quality fishery wastes which do not support high breeding

performance (Grémillet *et al.* 2008). Moreover, it is not clear if some individuals specialize on fishing discards while others rely more on 'natural' available prey, or if birds use discards opportunistically to complement a more heterogeneous and natural diet.

For the aquatic community, longline fisheries targeting top marine predators (tuna, billfishes and sharks) have been demonstrated to have profound effects on food web structures (Kitchell *et al.* 2002; Schindler *et al.* 2002; Heithaus *et al.* 2008). Globally, pelagic longline fisheries discard 28.5% of the total catches, or an estimated 506,481 tonnes annually (Kelleher 2005). A substantial proportion of discards in this fishery are 'finned' sharks (Kelleher 2005). In Brazil, sharks are an important target of pelagic longliners sold for the domestic market, and frequently compose the bulk of captures (Mayer & Andrade 2005; Bugoni *et al.* 2008a). Sharks are headed and gutted at sea, which results in viscera being discarded overboard and available for seabirds (Bugoni *et al.* 2008b). Shark livers are soft, very edible to seabirds, lipid-rich, large in size, and float in sea water, making them the preferred food for scavenging seabirds attending longline fishing vessels, swallowed in large chunks or whole by the large seabirds or in small pieces by small ones (Bugoni *et al.* 2008b).

During recent decades there has been an exponential expansion in the use of naturally occurring stable isotopes of nitrogen (ratio of $^{15}N/^{14}N$, expressed as $\delta^{15}N$) and carbon (ratio of $^{13}C/^{12}C$, expressed as $\delta^{13}C$) to address ecological questions (Lajtha & Michener 1994; Fry 2006). In studies of feeding ecology, trophic levels (TLs) can be determined by both carbon and nitrogen stable isotopes as has metabolic processes discriminate against the heavy isotope (isotopic fractionation), ultimately causing stepwise enrichment with increasing TL (Minagawa & Wada 1984). However, nitrogen is preferred for TL determination as it has been reported to differ by ~3 to 5‰ per TL, while carbon is enriched by about 1‰ (Minagawa & Wada 1984; Peterson & Fry 1987; Forero & Hobson 2003, but see Aberle & Malzahn (2007) for divergent enrichment values in low TLs). On the other hand, carbon can be used to discriminate between dietary sources, such as benthic vs pelagic, inshore vs offshore, and aquatic vs terrestrial (Peterson & Fry 1987; Forero *et al.* 2004; Hobson 2005a; Fry 2006). However, different tissues are renewed at different rates, providing archives of a number of temporal resolutions in a single organism (Hobson 2005a; Graham *et al.* 2007). For instance, bird feathers are metabolically inert tissues, thus representing the diet during the feather growing period, which could have occurred months or years ago, while blood cells have turnover rates of up to a few weeks (Hobson 2005b). Isotopic measurements provide an independent way of evaluating diet that supplements stomach content analysis, providing a time-integrated measure of food sources. In several studies of seabird communities, wide differences in isotopic signatures have been found between seabird species, including

wide differences in diets (e.g. Rau *et al.* 1992; Buckman *et al.* 2004; Kojadinovic *et al.* 2008), indicating the classical pattern of ecological niches of species.

In southern Brazil, studies of aquatic communities using stable isotopes have started recently on algae, plants and benthic invertebrates (Abreu *et al.* 2006) and fish (Garcia *et al.* 2007), but the offshore communities and higher TLs have not been addressed. Further south in the Patagonian region, the structure of the coastal seabird community has been studied on breeding grounds, and considerable overlap among species found (Forero *et al.* 2004), similar to Antarctica where tissues of top predators (fish, seabirds and seals) show great overlap in carbon and nitrogen isotope signatures, because the diet relies on a few species (Hodum & Hobson 2000). These studies suggested that coexistence of species in the community is possible as a consequence of superabundance of food or species diversification in morphology and foraging strategies. The identification of individual differences in diet or feeding ecology within populations could be of crucial importance to understanding population dynamics, and age and sex are potential factors influencing such differences (Forero & Hobson 2003).

In this study we analyzed stable isotopes in blood of Procellariiformes (albatrosses, petrels, shearwaters and storm-petrels) attending pelagic longline fishing vessels off southern Brazil, in tissues of potential food sources and discards from the fishery. We hypothesize that if discards from longline fishery are the main food source for the community, TLs would be similar and overlapping and isotopic signatures characteristic of discard would be identified in bird tissues. In contrast, a multi-level trophic-structured seabird community should be anticipated if species rely heavily on 'naturally' available prey, given the wide range of body sizes, from storm-petrels to greater albatrosses, and diverse foraging strategies (e.g. surface seizing, dipping, pursuit diving, plunging, scavenging). Furthermore, because the community is composed by migrants from southern latitudes (e.g. Patagonia, Antarctica, sub-Antarctic islands) which feed on prey with characteristic low carbon signatures and low TL (Quillfeldt *et al.* 2005; Cherel & Hobson 2007), a change in values of stable isotopes of carbon and nitrogen (or TL) in seabird blood could be detectable if discards from longline fisheries are an important food source. The use of stable isotope techniques is particularly relevant in this case because it integrates dietary information during a medium-term period rather than a snapshot of the diet provided by direct observations of feeding birds or stomach content methods (Barrett *et al.* 2007). A number of recent studies have inferred winter diet of seabirds based on stable isotopes in feathers grown during this period, and sampled afterwards in breeding grounds (e.g. Cherel *et al.* 2000, 2006; Quillfeldt *et al.* 2005). We had a different approach by sampling seabirds at sea during wintering period and aim to contribute a better understanding of seabird ecology during this period of their life-cycles

through direct at sea sampling of blood for stable isotope analysis and observations of birds feeding on discards.

MATERIAL AND METHODS

Study area

In the southwestern Atlantic Ocean cold waters of the Falkland (Malvinas) Current flowing northward meet the warm waters of the Brazilian Current flowing southward (Garcia 1998). The presence of highly productive waters from the Falkland (Malvinas) Current in winter sustains an important pelagic longline fishery in southern Brazil (Castello 1998), as well as the most abundant and diverse seabird community in Brazil (Neves *et al.* 2006b, Chapter 1). The separation between Brazilian and Falkland (Malvinas) Currents varies seasonally, from 35.8 to 38.6ºS, forming a heterogeneous area between both currents up to 1000 km wide with many eddies and fronts (Olson *et al.* 1988). Coastal waters over the continental shelf are strongly influenced by freshwater outflow from the Patos Lagoon and la Plata River (Ciotti *et al.* 1995, Fig. 7.1). The continental shelf in southern Brazil is wide (100-280 km), with gently sloping gradient (2 m km^{-1}), composed mostly of unconsolidated substrates, such as fine sand and mud (Calliari 1997), where a large trawling fleet operate for demersal species, mainly sciaenid fishes (Haimovici 1998; UNIVALI 2007).

Bird capture and sampling

Birds scavenging for offal and baits were attracted close to the vessel and captured using a cast net (Bugoni *et al.* 2008b, Chapter 2). In total 244 birds representing 14 species (Table 7.1) were captured on 58 trapping days from February to June 2006 and July to September 2007. Species sampled ranged from the 35 g storm-petrels feeding by pattering-dipping, to medium-sized diving shearwaters, to greater albatrosses (see Table 7.2 for body mass of birds and feeding techniques). Captures occurred during six cruises from 25 to 35ºS (Fig. 7.1), from vessels targeting Tuna (*Thunnus* spp.), Sharks (mainly Blue Shark *Prionace glauca*) and Swordfish (*Xiphias gladius*) using pelagic longline (Bugoni *et al.* 2008a, Chapter 9) and hook-and-line fisheries (Bugoni *et al.* 2008c, Chapter 10). Syringes and needles were used to collect 1 mL of blood from the tarsal vein, and birds were individually marked using metal rings to avoid resampling the same individual. Subsamples composed of two or three drops of whole blood were stored in 1.5 mL vials using absolute ethanol for molecular sexing and determinations of carbon and nitrogen isotope ratios.

Whole blood was chosen to trace recent diet as it should represent the food ingested during the last few weeks before sampling (Hobson 2005b). Another potential option was blood plasma as it reflects very recent diet, but because the turnover could be as short as one or two days for many species (Bearhop *et al.* 2003; Dalerum & Angerbjörn 2005),

results could be strongly influenced by the most recent meal rather than integrating dietary information from a more appropriate period (Dalerum & Angerbjörn 2005).

Samples of potential prey or food sources were collected when onboard vessels, and included squids and fish captured opportunistically by the vessel crew, baits used for the longline fishing (sardines, mackerel and squid), and tuna, sharks and Swordfish targeted by the vessels and whose remains were discarded. The inventory of potential prey was complemented in fishing industries at Rio Grande harbour by sampling demersal and coastal species from trawling fishery, and frozen fish collected during research cruises (Fundação Universidade Federal do Rio Grande, Table 7.1). Tissues of all birds and prey were preserved using absolute ethanol from the same brand (Merck® Darmstadt, Germany) and two different batches/bottles. Batches of this ethanol were found to have different carbon isotopic signatures (Bugoni *et al.* 2008d, Chapter 4). We are aware of the effects of ethanol on blood samples, but could not apply mathematical corrections proposed in Chapter 4 calculated based on batch 1 ($\delta^{13}C$ = -12.1‰) because the effects of ethanol with different signatures (batch 2, $\delta^{13}C$ = -24.3‰), on bird blood and on muscle of prey were not tested. However, $\delta^{13}C$ in blood of Cape (*Daption capense*) and White-chinned (*Procellaria aequinoctialis*) Petrels sampled in winter, i.e. without seasonal effect, and preserved using ethanol of both batches did not differ (t-test, t_{26} = 0.14, P > 0.89; and t_{15} = 2.08, P > 0.06, respectively), thus we follow the general assumption of other studies, that effects of ethanol with different signatures on samples are minimal, and did not affect conclusions.

Age determination

Pictures of bill and general plumage were taken from albatrosses and Giant Petrels for ageing. Giant Petrels change plumage colour from wholly dark brown to pale brown-greyish with age, which was used to identify juveniles from older birds. Black-browed Albatrosses change bill colour with age, which combined with wing moult allows age determination up to six years-old (Prince & Rodwell 1994). The same patterns of bill colour changes and moult were described for Gray-headed Albatrosses (*Thalassarche chrysostoma*) (Prince & Rodwell 1994). Ageing of Atlantic Yellow-nosed Albatrosses (*T. chlororhynchos*), which change from a wholly dark bill to orange at the culminicorn, ramicorn and ungues was used for ageing this species (Chapter 3). The relative age of Wilson's Storm-petrel (*Oceanites oceanicus*) was determined by molt patterns and foot web colouration (Quillfeldt *et al.* 2000). For all other species, birds not moulting during or after the breeding season were considered first year juveniles.

Wandering (*Diomedea exulans*) and Tristan (*D. dabbenena*) Albatrosses are virtually identical in plumage (Onley & Scofield 2007), so we used the discriminant function in Cuthbert *et al.* (2003a) to assign species. We further confirmed identification, after

sexing birds by molecular methods, using culmen length (see Cuthbert *et al.* 2003a for details).

Molecular sexing

Blood sub-samples were used for sexing birds after DNA extraction and PCR amplification of CHD genes using primers 2550F (Fridolfsson & Ellegren 1999) and 2757R (R. Griffiths, unpublished) and genes separated in 2% agarose gel by electrophoresis. Briefly, copies of CHD genes are present in both Z and W bird sexual chromosomes. CHD-Z and CHD-W genes differ in the base pair length of their non-coding regions. Because females are heterogametic (ZW) and males homogametic (ZZ), the separation of gene amplification products by size results in a single band for males and two bands for females. DNA extraction negative controls were included for every 23 samples. Positive and negative controls were included for each PCR reaction and 1/3 of all DNA extractions were repeated to confirm gender assignment. Furthermore, we used samples of previously sexed birds as controls: one male Atlantic Yellow-nosed Albatross killed in fisheries and sex determined by necropsy, and four ringed Wandering Albatrosses which were of known sex from observations at the breeding colony (A. Wood and R.A. Phillips, British Antarctic Survey, *in litt.*).

Sample analysis

The excess of ethanol in samples was removed and all blood samples were freeze-dried, ground and homogenised. Samples of 0.6 to 0.7 mg of blood were placed in tin capsules (4x6 mm) and analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech Elemental Analyser (Costech ECS 4010, Milan, Italy) coupled to a Thermo Finnigan Delta Plus XP Mass Spectrometer (Thermo Finnigan, Bremen, Germany). The analysis consists in measuring the ratio of the heavy and light isotopes of carbon and nitrogen in a sample and compares this to a standard. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards Pee Dee Belemnite limestone (carbon) and atmospheric air (nitrogen), according to the following equation:

$$\delta X = \left[\left(R_{sample} / R_{standard}\right) - 1\right] \times 1000 \qquad \text{(equation 7.1)}$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Three laboratory standards were analysed for every 10 unknown samples in each analytical sequence, allowing instrument drift to be corrected. Samples were shuffled in the analytical sequence according to individuals to minimize drift with time (Jardine & Cunjak 2005). For prey samples we follow recommendations of Becker *et al.* (2007) and collected muscle from the lateral region of fish and the mantle of squids and extracted

lipids. Because lipids are depleted in ^{13}C in relation to the whole tissues, we extracted them for carbon isotope analysis and used a non-extracted subsample for nitrogen determination (Schlechtriem *et al.* 2003; Sotiropoulos *et al.* 2004). Lipids were extracted from prey samples using the Soxhlet extraction technique with petroleum-ether as solvent (Schlechtriem *et al.* 2003), dried in oven, and subsequently ground, homogenized and encapsulated in the same way as blood samples. Based on internal standards (tryptophan), the analytical precision (± 1 SD) was estimated as ± 0.18‰ and ± 0.17‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Data analysis

Generalized Linear Model (GLM) was used to investigate the effects of different factors on the variation in $\delta^{13}C$ and $\delta^{15}N$ values. Factors used as explanatory variables for the model were species, with 14 levels (see Table 7.1), sex (2 levels - male, female), season (3 levels - summer, autumn, winter) and age (2 levels - first year juveniles and > 1 year-old). Because the distributions of residuals of stable isotope values were not normal, values of $\delta^{15}N$ were log transformed, and $\delta^{13}C$ values were $\log[n\times(-1)]$ transformed to account for negative values.

A simple model for trophic level (TL) of each seabird species (Forero *et al.* 2004) was calculated as:

$$TL_{bird} = \lambda + \left[\delta^{15}N_{bird} - \left(\delta^{15}N_{flyingfish} + \Delta_n\right)\right] / \Delta_n \qquad \text{(equation 7.2)}$$

where λ is the TL of flyingfish (*Cypselurus* sp.), assumed to be 3.0 (Kitchell *et al.* 2002), $\delta^{15}N_{fish}$ is the mean nitrogen isotopic ratio of flyingfish sampled off Brazil (8.5‰, Table 7.1), and Δ_n is the nitrogen isotopic enrichment factor between fish muscle and seabird blood (3.2‰, averaged from values in Hobson & Clark 1993; Bearhop *et al.* 2002 and Cherel *et al.* 2005). Post (2002) emphasizes that the choice of an appropriate baseline is one of the most important decisions in the application of stable isotopes to trophic studies, and that a good baseline should integrate spatially and temporally the isotopic variations of upper trophic levels. Flyingfish are an adequate baseline because they are common around the year at the shelf break and offshore waters where seabirds were sampled, and are prey of several upper level predators, including seabirds, tuna, billfishes, and sharks. Thus, a bird with diet composed exclusively by flyingfish will have the $TL_{flyingfish}$ + 1, and substituting parameters in the equation 7.2, the model becomes:

$$TL_{bird} = 4.0 + \left[\delta^{15}N_{bird} - (8.5 + 3.2)\right] / 3.2 \qquad \text{(equation 7.3)}$$

For non-avian components of the community, Forero *et al.* (2004) modified the parameter of isotopic fractionation (3.3‰, Rau *et al.* 1992) to account for whole body or muscle (vs blood), and expected to be typical of temperate food-webs in southern hemisphere. However, the δ^{15}N isotopic fractionation we used in the model (3.2‰) is close to the 3.3‰ reported by Rau *et al.* (1992) and to the 3.4‰ for more complex food-webs with several TLs (reviewed by Post 2002), thus we used the same parameters for calculations of TLs of seabirds and non-avian components of the community.

In order to determine if the seabird community was structured according to body mass of bird species, we tested the correlation between body mass obtained from mean values of males and females from Brooke (2004) and TL (Spearman rank correlation). To determine differences in TL according to feeding techniques we summarize the typical technique of each species (from Harper *et al.* 1985; Brooke 2004), and observed, throughout the sampling period, the feeding behaviours of birds behind longliners and size of discards swallowed by each species (Table 7.2).

Temporal variations in carbon and nitrogen signatures were tested for particular species grouping individuals according to season (Mann-Whitney test), or expressing days after 1 January for Pearson linear correlation. The δ^{13}C and δ^{15}N values of seabirds and in prey tissues were simultaneously compared by multivariate analysis of variance (MANOVA) with Wilk's lambda statistic. When significant differences were found, Tukey HSD test was used for *post hoc* comparisons between pairs. Values are presented as mean ± one standard deviation, and significance at $P < 0.05$, except otherwise indicated.

RESULTS

Isotopic signature in blood of seabirds

Mean carbon isotope values in blood of the 14 seabird species varied from -19.4‰ in Cape Petrel to -15.6‰ in Sooty Shearwater (*Puffinus griseus*) (Table 7.1), with values largely overlapping (Fig. 7.2 & 7.3). Despite the overlap there was significant effect of species on the combined carbon and nitrogen signatures (MANOVA, Wilk's lambda = 0.068, $F_{78, 812} = 29.4$, $P < 0.0001$). Characteristic low carbon values were found in some individuals of species that breed in Antarctic and sub-Antarctic regions, such as the first Cape Petrel attending the vessel (and sampled) in mid April, which had δ^{13}C = -23.2‰ and another specimen sampled in August with δ^{13}C = -23.5 (Fig. 7.3). Other species with some individuals presenting distinct low carbon values were Wandering Albatrosses, White-chinned Petrels and Wilson's Storm-petrels. δ^{13}C values in blood of the White-chinned Petrel formed two distinct groups of individuals, corresponding to birds sampled during autumn and winter (values less than -19‰ and higher than -19‰, respectively (Fig. 7.3) (Mann-Whitney test W = 371.5, $P < 0.007$). Difference in δ^{15}N values of these two seasonal groups was marginally non-significant (Mann-Whitney W = 350.0, P =

0.082). The temporal change in diet towards higher TLs was also evident in correlations for both carbon (Pearson correlation r = 0.81, df = 28, $P < 0.0001$) and nitrogen (r = 0.53, df = 28, $P = 0.002$). Similar correlations were also found for Black-browed Albatross for carbon (r = 0.52, df = 31, $P = 0.002$), but not for nitrogen (r = 0.19, df = 31, $P = 0.31$). Correlation for the Atlantic Yellow-nosed Albatross was not significant for either carbon or nitrogen (r = -0.1 and 0.01, both df = 31, $P = 0.57$ and $P = 0.98$, respectively). For the Spectacled Petrel (*Procellaria conspicillata*), correlations were significant, but negative for both carbon (r = -0.34, df = 0.03, $P = 0.03$) and nitrogen (r = -0.72, df = 36, $P < 0.0001$).

Nitrogen values in seabird blood were more variable than carbon values, and again with great overlap between most species (Figs. 7.2 & 7.3), despite significant effect of species (MANOVA and GLM, see below). The variation in $\delta^{15}N$ was large in all species with large sample sizes (Fig. 7.3). Mean values ranged from 9.6‰ in Cory's Shearwaters (*Calonetris diomedea*) to 16.4‰ in Southern Giant Petrels (*Macronectes giganteus*) (Table 7.1). Black-browed, Atlantic Yellow-nosed and Tristan Albatrosses also had high mean values. Cory's Shearwater, the only species that did not attend fishing vessels for discards, had lower nitrogen values in comparison with all other species (Tukey's test, all paired comparisons $P < 0.04$, Fig. 7.3 & 7.4). Species with some individuals displaying unusually low $\delta^{15}N$ were Cape Petrel (the same birds with low carbon values mentioned above), Wilson's storm and White-chinned Petrels, and Great Shearwater (*Puffinus gravis*). Closely related species did not differ in $\delta^{15}N$ (Tukey HSD test) and consequently in TLs (White-chinned vs Spectacled Petrels; Tristan vs Wandering Albatrosses; Black-browed vs Atlantic Yellow-nosed Albatrosses).

The GLM explains a significant proportion of the variation in $\delta^{13}C$ values, with three significant factors: species explained 51.9% ($F_{13, 227} = 15.0$, $P < 0.001$), followed by season and age, which despite being statistically significant, explained only 7.3% and 1% of the variation, respectively (season: $F_{2, 227} = 18.1$, $P < 0.001$; age: $F_{1, 227} = 5.3$, $P = 0.02$). Sex was a factor not significant in the model ($F_{1, 227} = 0.04$, $P = 0.84$). After simplifying this full model by removing non-significant factors (i.e. sex) the new model explained the same amount of variation in the dataset (60%) with all factors significant at $P < 0.05$. For $\delta^{15}N$, the model had a weaker performance explaining only 37.2% of the variation in values, again mainly due to species (32.8%, $F_{13, 227} = 9.2$, $P < 0.001$), followed by season ($F_{2, 227} = 5.6$, $P = 0.004$). The remaining factors were not significant (age: $F_{1, 227} = 3.2$, $P = 0.08$; sex: $F_{1, 227} = 0.07$, $P = 0.79$). A refined model for $\delta^{15}N$ without age and sex also explained only 37% of the variation, with remaining factors (species and season) significant at $P < 0.05$. Given these results, data for both sex and age classes were pooled for subsequent analysis.

Isotopic values of seabird food sources

Species of birds and their prey differed significantly in their combined (δ^{13}C and δ^{15}N) isotopic signatures (MANOVA, Wilk's lambda = 0.068, $F_{78, 812}$ = 29.4, P < 0.0001).

Mean δ^{13}C values in food resources potentially used by seabirds varied in tissues of large pelagic predators, from -17.4‰ to -15.6‰ in Blue (*Prionace glauca*) and Mako (*Isurus oxyrynchus*) Sharks, respectively, with tuna values in between (Table 7.1). Small pelagic fishes and squid, which also included baits used in the pelagic longline fishery (sardines, mackerel and squid) varied from -18.5‰ in the Chub mackerel (*Scomber japonicus*) to -16.0‰ in Cutlassfish (*Trichiurus lepturus*) (Figs. 7.4 & 7.5). This category included Mullet (*Mugil* sp.) with δ^{13}C value of -12.4‰, a characteristic coastal signature. Similar ranges of δ^{13}C values were found for demersal fish, from -16.6‰ in King Weakfish (*Macrodon ancylodon*) to -15.0‰ in Bluewing Searobin (*Prionotus punctatus*). Categories of prey (large pelagic fish, small pelagics, demersal fish and seabirds differed in combined δ^{13}C and δ^{15}N isotopic signatures (MANOVA, Wilk's lambda = 0.42, $F_{6, 76}$ = 6.9, P < 0.0001). However, One-way ANOVA with *post hoc* Tukey HSD test demonstrated the limited ability of δ^{13}C to discriminate among prey categories, only differing between seabird and demersal fish.

The range of δ^{15}N values in prey was considerably larger than δ^{13}C. Nitrogen isotope ratios in large pelagic predators ranged from 10.2 to 13.9‰ in small Bigeye Tuna (*Thunnus obesus*) and Yellowfin Tuna (*Thunnus albacares*), respectively (Table 7.1). Large Bigeye Tuna showed higher isotopic signature (12.0‰) than small tuna. The small pelagic species ranged considerably in isotopic values, from 6.4‰ in Filefish (*Aluterus monoceros*) to 16.8‰ in Cuttlassfish. Demersal fish had higher and less variable δ^{15}N values, with all averages between 15 and 17‰. Seabird tissues had δ^{15}N values differing from small pelagic species, but not from other prey categories. Small pelagics and demersal fish also differed in δ^{15}N values (Fig. 7.4 & 7.5).

Pelagic food web and trophic structure of the seabird community

Trophic level of pelagic seabirds in the southwestern Atlantic Ocean as derived from δ^{15}N values and trophic model indicates great overlap among seabird species (Table 7.1, Fig. 7.6). Cory's Shearwater had lower TL (mean 3.4) in comparison with all other species, which varied from 4.3 in the Wilson's Storm-petrel to 5.5 in the Southern Giant Petrel. All seabird species regularly attending fishing vessels had high TLs, including small species such as Cape Petrel and Southern Fulmar (*Fulmarus glacialoides*). The food web was not size structured (body mass vs TL; Spearman rank correlation r_s = 0.43, P = 0.13, n = 14 species; similar if removed species with sample sizes lower than 9, r_s = 0.44, P = 0.24, n = 9), or structured according to feeding techniques typical of the species, or the way

species scavenge for discards behind longline vessels (Table 7.2). Partitioning of discards was observed according to size of discards and size of bird species, aggressiveness and diving ability (Table 7.2). The preferred shark liver is usually discarded whole, but quickly fragmented by birds pecking this soft tissue. Shearwaters (*Puffinus* spp.) and less frequently *Procellaria* petrels and *Thalassarche* albatrosses, dive for discards, but could feed at the surface when competition is low. Greater albatrosses and Giant Petrels feed exclusively at the surface, with the latter fragmenting discards by pecking the liver, while the former swallows large chunks. Even species with soft bill as Cape Petrel are able to remove pieces of shark liver, or compete for small fragments with other species. Wilson's Storm-petrels wander among flocks feeding on tiny fragments. Flocks of Cory's and Cape Verde (*Calonectris edwardsii*) Shearwaters were frequently observed during summer and autumn roosting nearby and feeding on flyingfish and squids when prey were driven to the surface by tuna, but do not attend vessels for discards.

TL of demersal fish species was on average higher than TLs of seabirds (Table 7.1, Figs. 7.5 & 7.6), with some species at the same TL as seabirds (Fig. 7.6). Large pelagic predators were one TL lower than seabirds (except Cory's Shearwater, Fig. 7.4), followed by small pelagics. TL of components of the 'small pelagics' category was heterogeneous, with species differing by up to 3.5 TLs. Bentho-pelagic species arbitrarily assigned to this group such as Cutlassfish, Rough scad (*Trachurus lathami*) and Short-finned squid (*Illex argentinus*) used as bait, ranked high. Somewhat unexpected, Argentine anchovy had mean TL 4.9, while both squid groups (*Illex* used as bait vs *Illex*/*Ommasthrephes bartrami* captured by fishermen from the longline vessels, simultaneously with seabird samplings) differed by 1.5 TLs.

DISCUSSION

Procellariiformes wintering in the southwestern Atlantic Ocean had undergone a marked shift in their isotopic signatures of carbon and nitrogen in blood in comparison to data from breeding grounds and have TLs greatly overlapping. During the breeding period these birds feed on prey with low carbon isotopic values typical of high and temperate latitudes (Quillfeldt *et al.* 2005; Cherel *et al.* 2006). Similarly, their crustacean, squid and small fish prey have lower nitrogen isotopic values and low TL, but birds shift to a diet enriched in δ^{15}N derived from top predators when on SW Atlantic wintering grounds. Isotopic values of nitrogen and carbon in seabird blood relative to main food resources suggest a remarkably strong dependence on discards from the pelagic longline fishery. Isotopic signatures of seabird tissues are on average higher than those of large pelagic predators, and lower than demersal fish, suggesting that fishery waste from pelagic longline is the main food source for all seabird species, except Cory's Shearwater. The reliance of the seabird community on fishery wastes from pelagic longliners is confirmed by nitrogen values 3-4‰ lower in Cory's Shearwater than in other seabirds. This species

was simultaneously present in the area, but the only one feeding on small pelagic prey instead of discards. Moreover, the dependence of bird species on the same food resource is postulated to be the primary cause of the unstructured food web with great overlap in TL between species and individuals. This implies scramble competition for an abundant food resource, rather than different ecological niches for each seabird species.

Methodological considerations

Seabird species sampled in the current study are representative of the community of pelagic seabirds in the SW Atlantic Ocean as determined by census (Neves et al. 2006b; Bugoni et al. 2008d, Chapter 8). A few relatively frequent but not abundant species, such as the Atlantic Petrel (*Pterodroma incerta*) and the Cape Verde Shearwater, but which do not attend vessels for discards, could not be sampled, yet all abundant species year round were included. In addition, because only birds attending vessels were sampled, there is a possibility that only birds specialized in discards were sampled. However, because individual birds remain for short periods attending the same vessel, as observed through banded birds or those with diagnostic external marks, and due to the high flying ability of albatrosses and petrels, we are confident that the sampling is representative of the community in the area.

Knowledge of seabird diet has been based on conventional methods during the breeding period (Duffy & Jackson 1986; Hobson *et al.* 1994; Barrett *et al.* 2007). More recently, stable isotope analysis has been shown to be a useful complementary approach to stomach content analysis, which can document the feeding ecology during wintering period through sampling of feathers grown when birds are away from breeding grounds. This study is, to our knowledge, the first to document the diet of a wintering seabird community sampling tissues at sea, and coupled with direct feeding observations. Conventional methods for dietary studies are known to be biased by the more rapid digestion of soft-bodied prey and retention of hard body parts (e.g. squid beaks and fish otoliths, Bugoni & Vooren 2004; Barrett *et al.* 2007). Similar to gelatinous prey which were considered as minor prey (see Cherel & Klages 1997 for a review of food items of albatrosses), but more recently recognized as being ingested frequently (e.g. Catry *et al.* 2004), viscera of large predatory fish and shark liver in particular, are expected to be digested rapidly and are probably undetected in studies using traditional methods. Even stomach content analysis of birds killed by longline fisheries in the SW Atlantic failed to report these items (Colabuono & Vooren 2006). We have shown both by direct observation and stable isotope analysis, that shark liver and remains of other large pelagic fish are the main food source of albatrosses and petrels during the wintering period in southern Brazil. Shark livers discarded in other fisheries around the world are also expected to provide food for albatrosses, petrels and shearwaters, except those where shark finning is practiced.

Wintering vs breeding diet as revealed by stable isotopes

The carbon and nitrogen values in SW Atlantic seabirds are on average higher than in tissues of the same species from breeding grounds in Patagonia and Antarctica (Hodum & Hobson 2000; Forero *et al.* 2004; Quillfeldt *et al.* 2005; Cherel *et al.* 2006) as expected for carbon values from this lower latitude (Quillfeldt *et al.* 2005; Cherel & Hobson 2007) and for nitrogen isotopic values from higher TLs (e.g. Hobson et al. 1994; Forero *et al.* 2004). More interesting, however, is the marked change in diet reflected by nitrogen and carbon isotope signatures and the increase in TL that occurred in all species, except Cory's Shearwater. During breeding, Cory's Shearwater feed on similar epipelagic and epimesopelagic fish and squid, and have similar δ^{15}N values in blood to those found in the wintering area (range 10-13‰, Navarro *et al.* 2007). All other species are migrants from southern latitudes, and when they were sampled early in the season, probably soon after arriving (e.g. Cape, Wilson's Storm and White-chinned Petrels, Fig. 7.3), and banded Wandering Albatrosses rearing chicks in South Georgia when sampled in our area (Bugoni *et al.* 2008b), still have carbon and nitrogen signatures typical of their breeding grounds. From the Figure 7.5 is evident that more negative δ^{13}C values carried from southern breeding grounds are influencing the mean and standard deviation shown. It would be expected to find some species commuting between coastal and pelagic waters, as the same seabirds occur over inshore waters. A large source of nutrients supplied by the freshwater outflow from Patos Lagoon and la Plata River influences the coastal phytoplankton biomass (Ciotti *et al.* 1995), which results in a carbon isotope value typical of coastal nektonic fish, such as the mullet (Fig. 7.4). However, this pattern was not found and signatures are typically from offshore region. For example, it has been demonstrated that Spectacled Petrels in the SW Atlantic Ocean stay over waters at the shelf break and offshore, spatially segregated from the more coastal White-chinned Petrel (Chapter 8). If this pattern was evident in δ^{13}C values, Spectacled Petrels would have more negative values (offshore) than White-chinned Petrel (inshore), but the opposite was found (Fig. 7.2). This was presumably due to White-chinned Petrels carrying more negative carbon signatures from sub-Antarctic breeding grounds. The inshore-offshore signature detected in other seabird communities (e.g. Hobson 1993; Cherel *et al.* 2006) and in mullets and other fishes here, was not found in albatrosses and petrels, which shown the typically offshore signature.

Seabird food sources and trophic structure

Some seabird communities are known to rely mainly on a single or a few food resources and so have overlapped TLs, for example krill (*Euphausia* sp.) in the diet of Antarctic and sub-Antarctic penguins and aerial seabirds (Croxall & Prince 1987; Hodum & Hobson 2000), anchovies (*Engraulis ringens*) in the diet of seabirds from the Humboldt Current system (Duffy 1983), capelin (*Mallotus vilosus*) in the diet of Newfoundland–Labrador

seabirds (Carscadden *et al.* 2002). Forero *et al.* (2004) found high degree of TL overlap in 14 breeding seabirds in Patagonia, with 13 species within 0.7 TLs. Overlap in TL is common where one or a few natural prey are the base food source of the seabird community. Pelagic seabirds studied here vary by 2 TLs, higher than the range reported previously for some other seabird communities (see review in Forero *et al.* 2004). Despite different studies reporting TLs are based in different assumptions, the variation found here reflects the diet from low TL of the Cory's Shearwater, which feeds on low TL pelagic flyingfish through albatrosses and petrels feeding on fishery discards from large predatory fish. However, the same magnitude of differences was found within species, particularly those migrating from regions where they occupy lower TLs. For example, TL of Cape Petrel varied from 3.3 to 5.4 and in White-chinned Petrel from 3.9 to 5.8.

The Subtropical Convergence Area in the SW Atlantic Ocean is an heterogeneous environment where a range of potential natural prey are present and expected to provide food for seabird with feeding strategies and body sizes as diverse as these studied here. In addition, a range of other fisheries operate over the adjacent continental shelf, the largest fleet being the trawling for shrimp and sciaenid fish and the gillnetting for fish (IBAMA/CEPERG 2007; UNIVALI 2007) with over 800 vessels. Trawling fishery is a potentially major source of undersized and non-commercial fish for seabirds (Haimovici *et al.* 1994; Haimovici 1998). Due to a more complex food web, demersal fish had δ^{15}N values higher than seabirds (Fig. 7.4, 7.5 & 7.6) and are probably not important in the diet of birds sampled here. A priori, we can not rule out this as food source for seabirds, as we have sampled demersal fish from commercial landings, and discards are mainly of small fish which could have lower δ^{15}N values (not represented in our sample). However, a coastal signature such as that shown in Figure 7.4 and Garcia *et al.* (2007) was not evident in seabird blood. In contrast to other seabird communities where overlap among species is driven by the availability of a few natural food resources, the overlap among the diverse community of seabirds, and the unstructured food web we reported, appears to results from anthropogenic activity, a monotonous diet shared among species, ages and sexes. In general, communities of vertebrates are expected to be size-structured. For example, Al-Habsi *et al.* (2008) found that a demersal fish community sampled by trawling was size-structured in terms of trophic level. However, Jennings *et al.* (2008) found that the heaviest predators rarely fed at the highest trophic level, as we also found here. It has been suggested that only a few seabird species in each community feed on fishery discards, and these tend to be large seabirds that can compete effectively at fishing boats for this resource (e.g. Hudson & Furness 1988; Votier *et al.* 2004). However, discards from pelagic longline fishery have been demonstrated to be a 'democratic' food source for seabirds with different foraging abilities, supporting birds across the entire size range, from storm-petrels to large albatrosses.

Costs and benefits of attending longline vessels: species-specific outcomes

Events occurring during the wintering periods are known to shape population dynamics of birds (Marra *et al.* 1998; Cherel *et al.* 2006; Robb *et al.* 2008). Whereas pelagic longline fisheries cause negative impacts on the aquatic community by removing top predators (Kitchell *et al.* 2002; Schindler *et al.* 2002; Heithaus *et al.* 2008), the effects on seabirds are variable. Albatrosses and petrels greatly benefit from pelagic longline discards, where the preferred shark liver is a lipid-rich and convenient food source. Birds feeding on beneficial discard during hauling operations can be incidentally killed when longline is deployed, and they get drowned by feeding on baited hooks (Bugoni *et al.* 2008a, Chapter 9). Globally, sixty-one seabird species are known to be caught in longline, mostly albatrosses and petrels (Brothers *et al*. 1999), the main cause of steep declines of 19 out of 21 albatross species currently listed as threatened (IUCN 2007). Small species are performing better (e.g. the Cape Petrel, Southern Fulmar, Wilson's Storm-petrel, Great Shearwater) because they are not able to swallow baited hooks, do not scavenge for baits or rarely swallow large pieces of food, such as the Southern Giant Petrel. Overall, the negative impacts of longline fishery on the ecosystem and on some albatrosses and petrels appear to be greater than the benefits for some abundant and widespread species.

Table 7.1. Stable-carbon and nitrogen isotope values (mean ± 1 SD, range) of Procellariiform seabirds and their potential food sources (prey, baits and fish discards) sampled at sea off Brazil.

Sample	n	$\delta^{13}C$ (‰) mean ± SD (range)	$\delta^{15}N$ (‰) mean ± SD (range)	Trophic level mean ± SD
Seabirds				
Cory's Shearwater *Calonectris diomedea*	2	-17.4 ± 0.0 (-17.4 -17.3)	9.6 ± 1.2 (8.8 -10.4)	3.4 ± 0.4
Wilson's Storm-petrel *Oceanites oceanicus*	3	-18.9 ± 1.1 (-20.1 -18.2)	12.8 ± 1.7 (11.3 -14.7)	4.3 ± 0.5
Southern Fulmar *Fulmarus glacialoides*	10	-18.5 ± 0.7 (-19.7 -17.1)	14.0 ± 0.9 (12.9 -16.0)	4.7 ± 0.3
Wandering Albatross *Diomedea exulans*	9	-19.3 ± 0.7 (-20.3 -18.2)	14.2 ± 0.5 (13.5 -15.1)	4.8 ± 0.2
Great Shearwater *Puffinus gravis*	37	-16.9 ± 0.5 (-18.5 -16.2)	14.4 ± 1.7 (11.0 -17.0)	4.9 ± 0.5
Cape Petrel *Daption capense*	31	-19.4 ± 1.2 (-23.5 -17.9)	14.5 ± 1.5 (9.5 -16.3)	4.9 ± 0.5
Northern Giant Petrel *Macronectes halli*	1	-18.8	14.6	4.9
Spectacled Petrel *Procellaria conspicillata*	38	-16.6 ± 0.9 (-18.3 -14.8)	15.1 ± 1.0 (13.8 -17.1)	5.1 ± 0.3
White-chinned Petrel *Procellaria aequinoctialis*	30	-18.0 ± 1.8 (-21.5 -15.9)	15.1 ± 1.9 (11.3 -18.2)	5.1 ± 0.6
Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*	33	-17.0 ± 0.5 (-17.8 -15.9)	15.4 ± 1.3 (13.9 -18.9)	5.2 ± 0.4
Sooty Shearwater *Puffinus griseus*	2	-15.6 ± 0.3 (-15.9 -15.4)	15.5 ± 0.4 (15.3 -15.8)	5.2 ± 0.1
Tristan Albatross *Diomedea dabbenena*	4	-17.6 ± 0.6 (-18.3 -16.9)	15.6 ± 0.6 (14.8 -16.0)	5.2 ± 0.2
Black-browed Albatross *Thalassarche melanophris*	33	-17.2 ± 0.6 (-18.2 -16.0)	16.3 ± 0.9 (14.7 -17.6)	5.4 ± 0.3
Southern Giant Petrel *Macronectes giganteus*	11	-17.4 ± 0.4 (-18.0 -16.8)	16.4 ± 1.3 (14.1 -17.7)	5.5 ± 0.4
Large pelagics (fishes and sharks)				
Swordfish *Xiphias gladius*	2	-15.6±1.9 (-16.9 -14.2)	13.4 ± 1.1 (12.6 -14.2)	4.5 ± 0.4
Yellowfin Tuna *Thunnus albacares*	4	-16.5±0.1 (-16.6 -16.4)	13.9 ± 0.8 (12.7 -14.5)	4.7 ± 0.3
Bigeye Tuna *Thunnus obesus* (large)	7	-16.9±0.2 (-17.3 -16.6)	12.0 ± 1.1 (10.4 -13.5)	4.0 ± 0.3

Table 7.1. (Continued)

Bigeye Tuna *Thunnus obesus* (small)	4	-16.5±0.6 (-17.1 -15.8)	10.2 ± 1.2 (8.8 -11.6)	3.5 ± 0.4
Skipjack Tuna *Katsuwonus pelamis*	3	-16.7±0.5 (-17.2-16.3)	12.4 ± 1.3 (11.0 -13.5)	4.2 ± 0.4
Blue Shark *Prionace glauca* (small and large)	14	-17.4±0.5 (-18.1-16.3)	12.9 ± 0.5 (12.4 -14.2)	4.4 ± 0.1
Mako Shark *Isurus oxyrhynchus*	1	-15.6	16.6	5.5
Dolphinfish *Coryphaena hippurus*	1	-17.2	13.4	4.5
Small pelagics (fishes and squid)				
Flying Fish *Cypselurus* sp.	6	-18.1 ± 0.4 (-18.6 -17.6)	8.5 ± 0.8 (7.0 -9.5)	3.0 ± 0.3
Brazilian Sardine *Sardinella brasiliensis* (bait)	7	-17.6 ± 0.6 (-18.3 -16.7)	11.4 ± 1.0 (10.5 -13.3)	3.9 ± 0.3
Rough Scad *Trachurus lathami*	9	-16.1 ± 0.1 (-16.4 -15.9)	16.1 ± 1.1 (14.6 -17.1)	5.4 ± 0.3
Cutlassfish *Trichiurus lepturus*	16	-16.0 ± 0.4 (-17.0 -15.6)	16.8 ± 0.9 (14.9 -18.0)	5.6 ± 0.3
Chub Mackerel *Scomber japonicus* (bait)	16	-18.5 ± 0.3 (-18.9 -17.8)	10.9 ± 0.4 (10.1 -11.4)	3.8 ± 0.1
Almaco Jack *Seriola rivoliana*	5	-16.8 ± 0.3 (-17.3 -16.4)	6.5 ± 0.9 (5.8 -8.0)	2.4 ± 0.3
Argentine Anchovy *Engraulis anchoita*	14	-16.5 ± 0.5 (-17.5 -15.9)	14.7 ± 0.5 (13.9 -15.9)	4.9 ± 0.2
Filefish *Aluterus monoceros*	6	-16.6 ± 0.3 (-17.0 -16.3)	6.4 ± 1.0 (5.0 -7.5)	2.4 ± 0.3
Mullet *Mugil* sp.	10	-12.4 ± 1.3 (-14.3 -10.6)	13.0 ± 0.9 (11.5 14.3)	4.4 ± 0.3
Short-finned Squid *Illex argentinus* (bait)	21	-17.2 ± 0.6 (-18.1 -15.8)	14.2 ± 1.6 (8.8 -15.9)	4.8 ± 0.5
Squid *Ommastrephes bartrami/Illex argentinus*	8	-16.7 ± 0.4 (-17.1 -16.0)	9.3 ± 0.8 (8.5 -10.7)	3.3 ± 0.2
Demersal fish				
White Croaker *Micropogonias furnieri*	11	-15.1 ± 0.6 (-15.9 -14.0)	16.5 ± 0.4 (15.9 -17.2)	5.5 ± 0.1
Striped Weakfish *Cynoscion guatucupa*	10	-16.1 ± 0.4 (-16.8 -15.8)	15.8 ± 0.8 (14.8 -16.8)	5.3 ± 0.2
King Weakfish *Macrodon ancylodon*	10	-16.6 ± 0.3 (-17.2 -16.1)	15.6 ± 1.1 (13.7 -16.7)	5.2 ± 0.3
Argentine Croaker *Umbrina canosai*	10	-15.5 ± 0.3 (-15.9 -15.1)	16.6 ± 0.8 (15.3 -17.5)	5.5 ± 0.2
Brazilian Codling *Urophycis brasiliensis*	11	-15.6 ± 0.5 (-16.2 -14.8)	16.5 ± 0.7 (15.3 -17.7)	5.5 ± 0.2
Wreckfish *Polyprion americanus*	1	-15.7	17.7	5.9

Table 7.1. (Continued)

Red Porgy *Pagrus pagrus*	1	-15.9	15.5	5.2
Pink Cusk-eel *Genypterus brasiliensis*	1	-16.1	14.7	5.0
Bluewing Searobin *Prionotus punctatus*	9	-15.0 ± 0.5 (-15.9 -14.1)	16.9 ± 0.7 (15.8 -17.6)	5.6 ± 0.2
Catfish (Ariidae)	9	-15.4 ± 0.5 (-16.1 -14.7)	17.0 ± 0.9 (15.4 -18.2)	5.6 ± 0.3

Table 7.2. Body size, trophic level, feeding method and partitioning of discards from longline fisheries by Procellariiformes in the southwestern Atlantic Ocean. NA – not applicable.

Species	Body mass (g)*	Trophic level	Feeding behaviour*	Attending longliners for discards	Feeding location/method	Size of discarded pieces ingested
Atlantic Yellow-nosed Albatross	2199	5.2	surface-seizing; shallow plunging	Yes	surface; diving; kleptoparasitism	large
Black-browed Albatross	3444	5.4	surface-seizing; shallow plunging	Yes	surface; diving; kleptoparasitism	large
Cape Petrel	464	4.9	surface-seizing; shallow plunging; filtering	Yes	surface	small
Cory's Shearwater	840	3.4	surface-seizing; pursuit plunging; pursuit-diving	No	NA	NA
Great Shearwater	834	4.9	surface-seizing; pursuit plunging; pursuit-diving	Yes	diving	small
Northern Giant Petrel	4313	4.9	Scavenger; surface-seizing	Yes	surface; kleptoparasitism	small
Southern Giant Petrel	4440	5.5	Scavenger; surface-seizing	Yes	surface; kleptoparasitism	small
Southern Fulmar	879	4.7	surface-seizing	Yes	surface	small
Spectacled Petrel	1191	5.1	surface diving; surface seizing	Yes	diving; kleptoparasitism	medium fragments
Sooty Shearwater	803	5.2	pursuit-diving	Yes	diving	medium

Table 7.2. (Continued)

Tristan Albatross	7050	5.2	surface-seizing	Yes	surface; kleptoparasitism	medium
Wandering Albatross	8730	4.8	surface-seizing	Yes	surface; kleptoparasitism	large
White-chinned Petrel	1335	5.1	surface diving; surface seizing	Yes	diving; kleptoparasitism	medium
Wilson's Storm-petrel	35	4.3	dipping-pattering	Yes	dipping; pattering	small

* From Harper *et al.* (1985) and Brooke (2004).

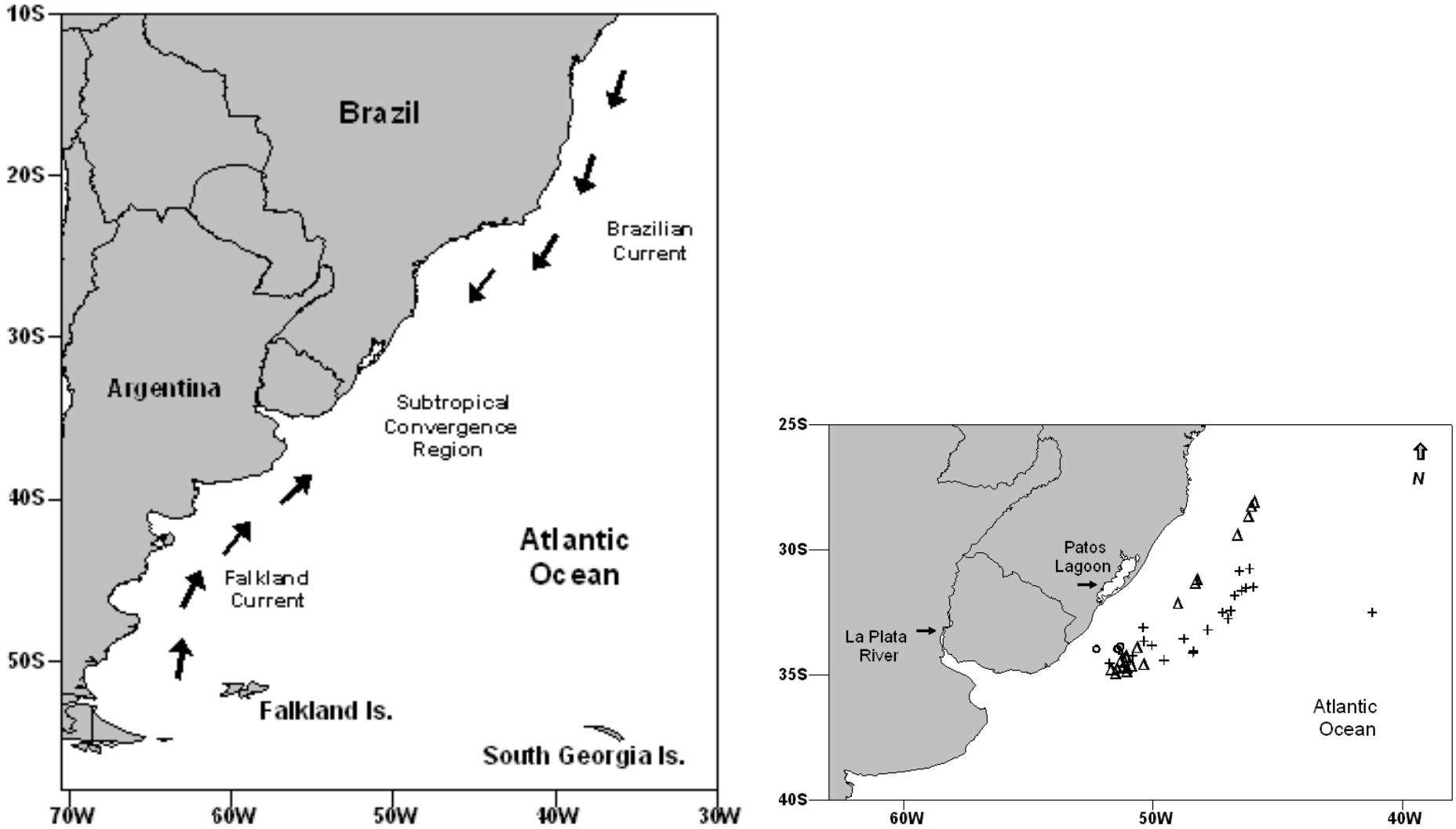

Figure 7.1. Map of the Atlantic Ocean off southern Brazil where seabirds were sampled in summer (○), autumn (Δ) and winter (**+**). A single location usually represents several sampled birds. The approximate location of the Subtropical Convergence Area and other localities mentioned in the text are shown.

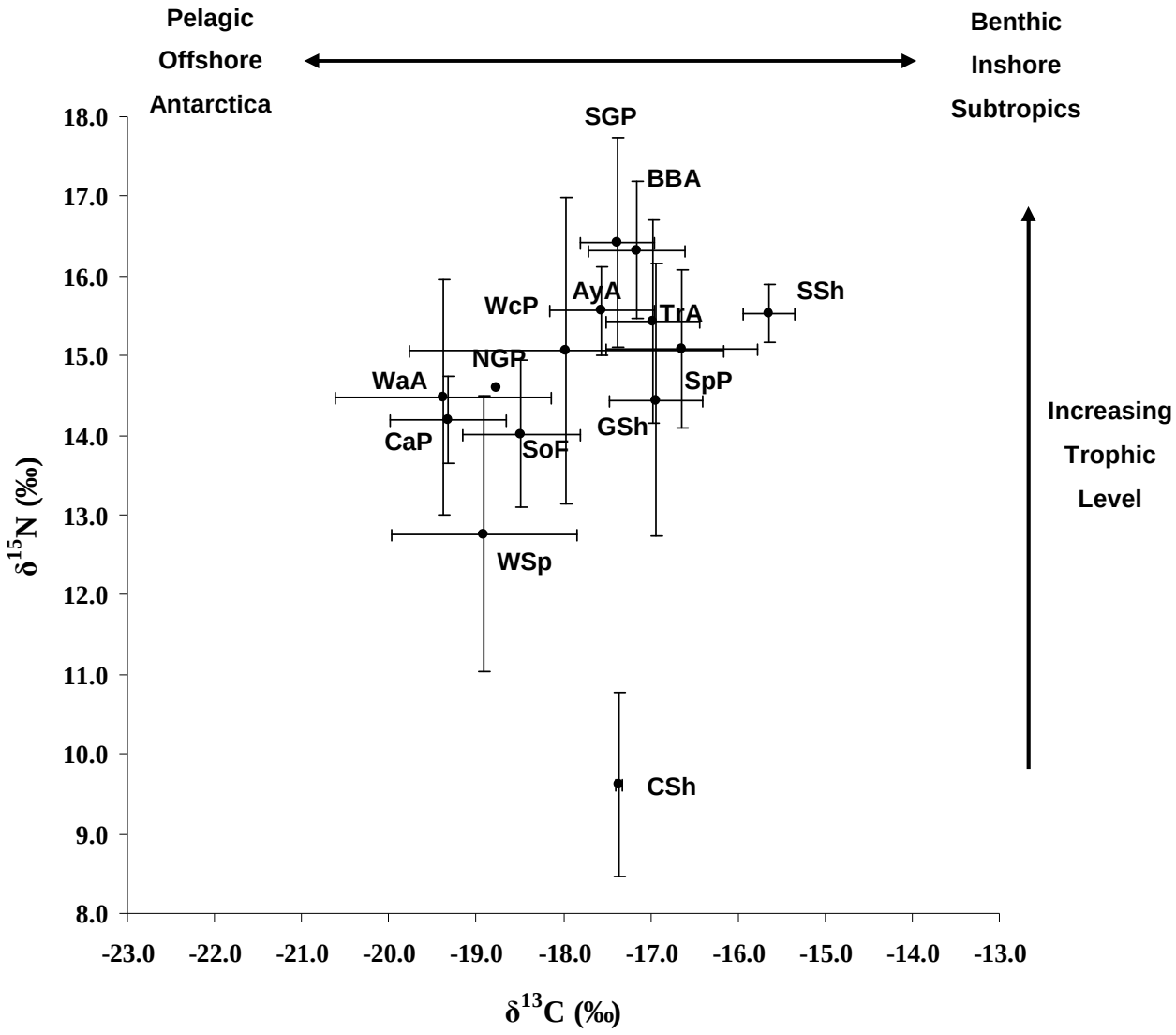

Figure 7.2. Mean ± 1 SD of stable carbon and nitrogen ratios in whole blood of albatross, petrel and shearwater species sampled at sea off southern Brazil. Species codes: CSh – Cory's Shearwater; GSh – Great Shearwater; SSh – Sooty Shearwater; WSp- Wilson's Storm-petrel; WcP – White-chinned Petrel; SpP – Spectacled Petrel; SGP – Southern Giant Petrel; NGP – Northern Giant Petrel; CaP – Cape Petrel; SoF – Southern Fulmar; BBA – Black-browed Albatross; AyA – Atlantic Yellow-nosed Albatross; WaA – Wandering Albatross; TrA – Tristan Albatross.

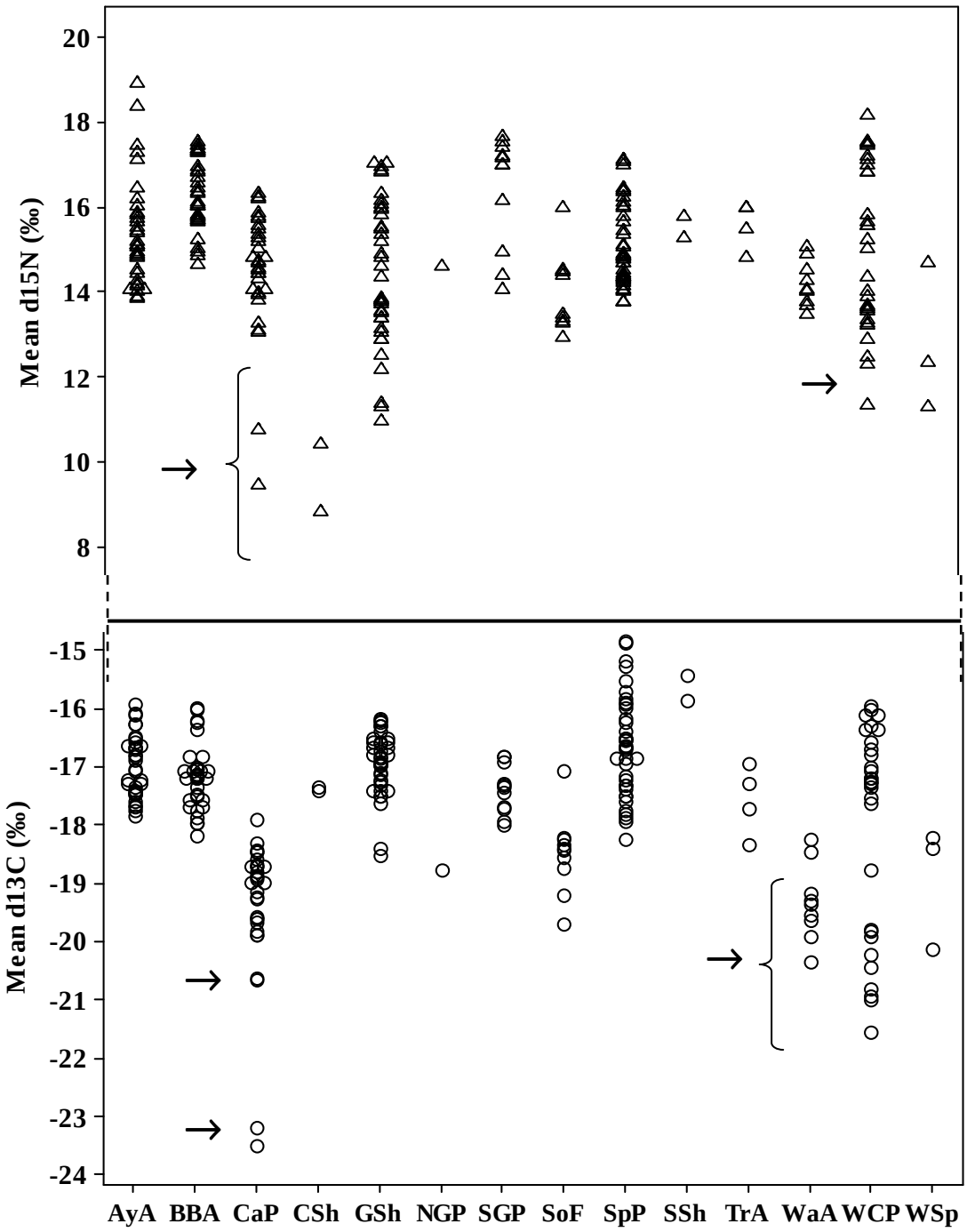

Figure 7.3. Plot of individual values of $\delta^{15}N$ (above) and $\delta^{13}C$ (below) of 244 seabirds sampled off Brazil. Arrows indicate individuals with signatures from Antarctic prey (carbon) and lower trophic levels (nitrogen) suspected to be southern migrants. Species codes as in Fig. 7.2.

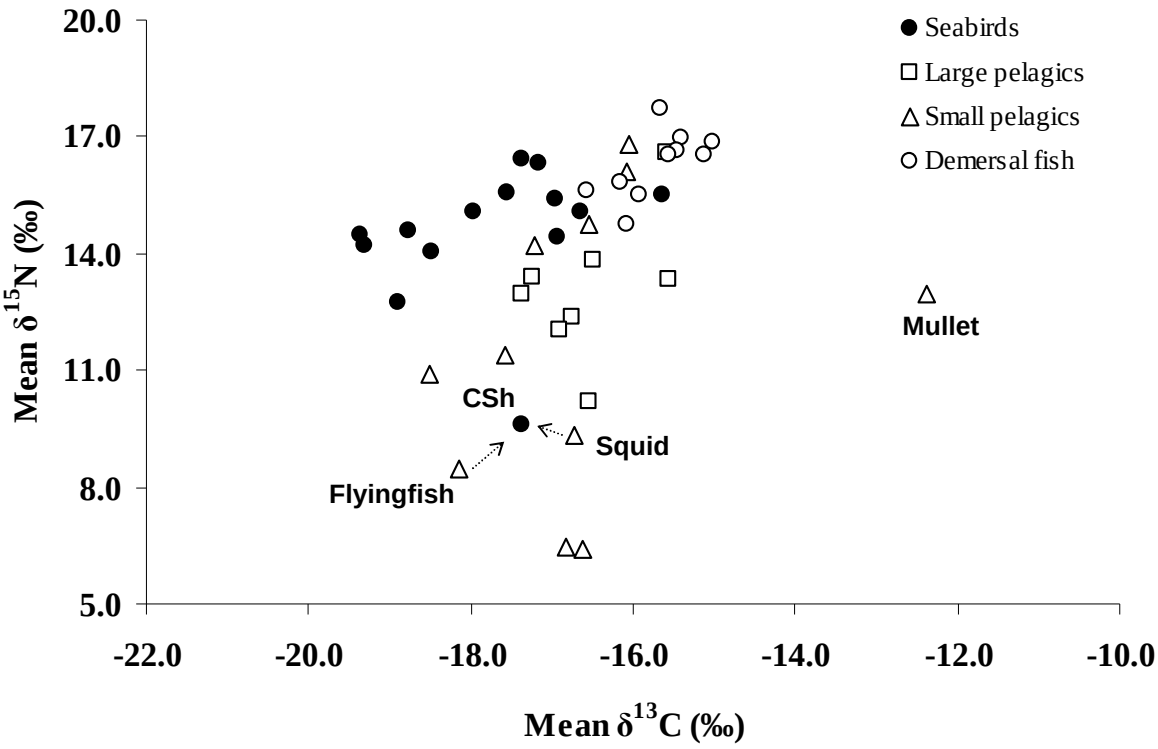

Figure 7.4. Mean stable carbon and nitrogen ratios of seabirds sampled at sea off southern Brazil and their potential food sources. SD bars omitted for clarity.

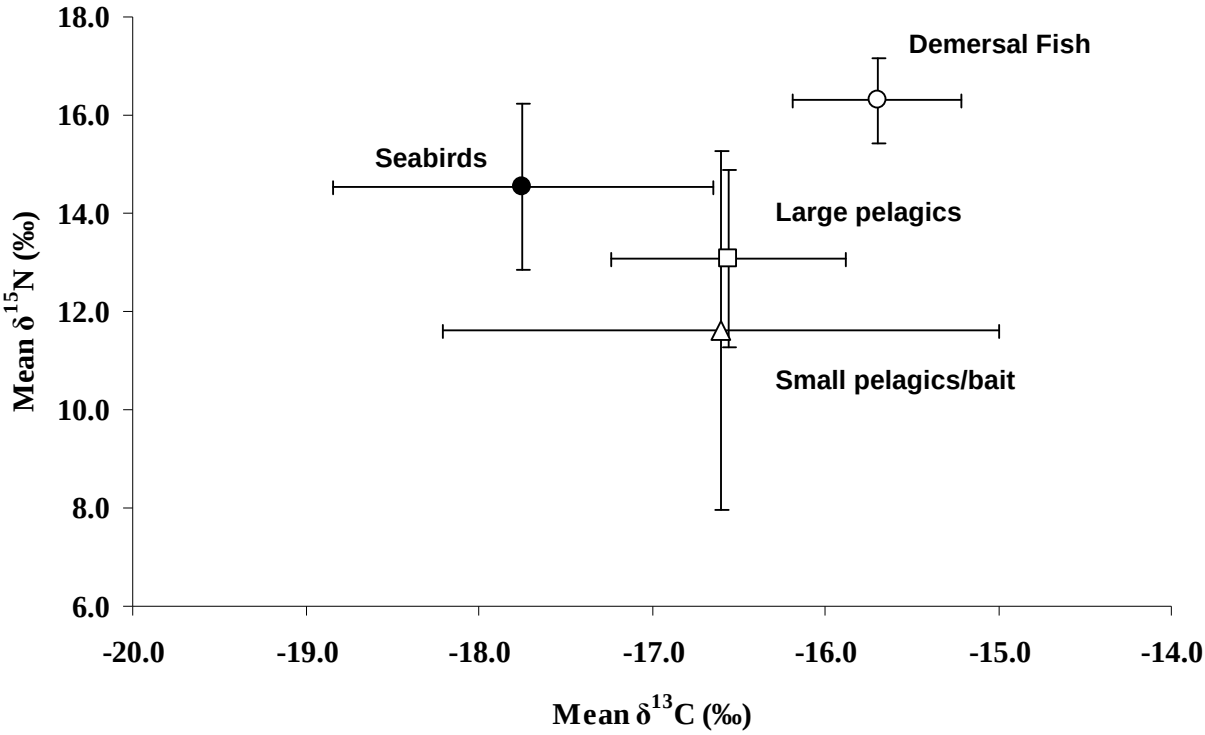

Figure 7.5. Mean ± 1 SD of stable carbon and nitrogen ratios of seabirds and their categories of food sources.

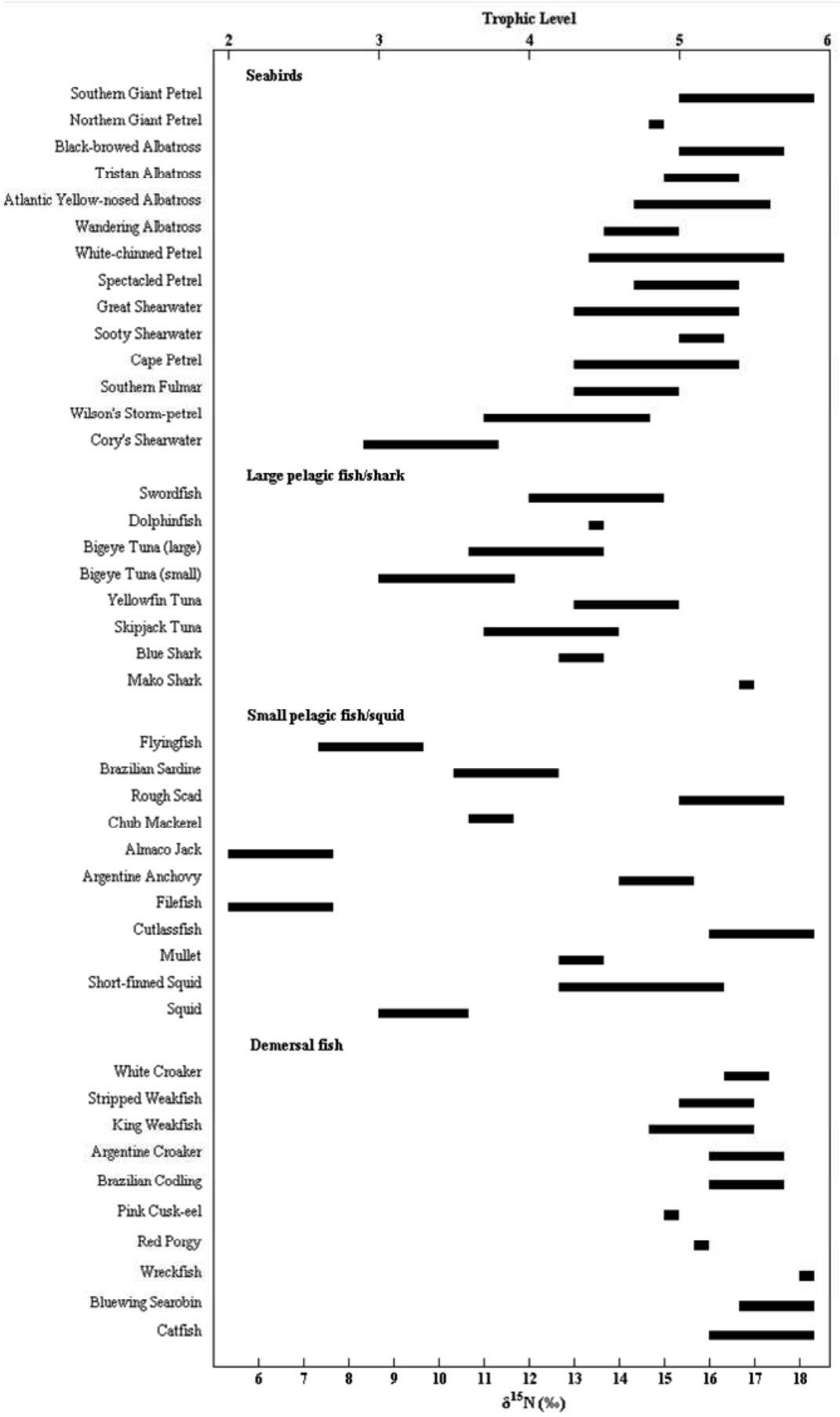

Figure 7.6. Trophic levels and $\delta^{15}N$ (‰) values ± 1 SD of albatrosses, petrels and shearwaters sampled at sea off southern Brazil and their potential food sources.

Chapter 8

MARINE HABITAT USE OF WINTERING SPECTACLED PETRELS *PROCELLARIA CONSPICILLATA* AND OVERLAP WITH LONGLINE FISHERY

Bugoni L, D'Alba L, Furness RW. Marine habitat use of wintering Spectacled Petrels *Procellaria conspicillata* and overlap with longline fishery. **Under review**

ABSTRACT

Satellite transmitters were used to determine the marine habitat utilization and fishery overlap of wintering Spectacled Petrels *Procellaria conspicillata* in the southwestern Atlantic Ocean in 2006 and 2007. Kernel density analysis of tracked birds demonstrated intense use of waters in the Brazilian Exclusive Economic Zone, from 26 to 31ºS, mainly over the continental shelf break and offshore waters (mean depth in the < 20% kernel density areas = 1043 ± 794 m), over warm tropical and subtropical (mean SST = 22°C and 21°C in 2006 and 2007, respectively) and mesotrophic/oligotrophic (chlorophyll *a* density 0.301 mg m^{-3} and 0.281 mg m^{-3} in 2006 and 2007, respectively) waters. The marine habitats used by Spectacled petrel and described by bathymetry, SST and productivity are remarkably different from those of the sister species White-chinned Petrel *P. aequinoctialis*, which occurs in the area during the winter, but remains over the continental shelf, on Sub-Antarctic and oligotrophic waters. A close association between birds and pelagic longline fishery was demonstrated through comparison of the main kernel areas used by Spectacled Petrels and the pelagic longline fleet, with fishing effort at resolution of 1° x 1° quadrants and complete temporal overlap between bird tracking and fishing period. Travelled speeds and distances during night and daytime periods did not differ. This study demonstrates the importance of high resolution fishing effort data to address relationships between bird marine habitat use and specific fishing fleets, and to determine marine habitats and investigate at sea segregation between closely related species.

INTRODUCTION

Longline fisheries are the most important global threat to the survival of most albatross populations (Gales 1997), also adversely affecting several petrel species (Weimerskirch *et al.* 1999; BirdLife International 2004). The threats frequently occur far from breeding grounds because Procellariiformes travel thousands of kilometres to feed during breeding and wintering periods (Weimerskirch *et al.* 1999; Phillips *et al.* 2006). Since the development of satellite technologies for tracking swans and eagles in 1983-84 (Seegar *et al.* 1996) and large seabirds such as the Southern Giant Petrel *Macronectes giganteus* (Parmelee *et al.* 1985), a range of detailed information on movements of albatrosses and petrels away from breeding colonies was generated. Further improvements in location accuracy and device miniaturization were achieved and, more recently, bird-borne satellite transmitters have been applied in the context of conservation of these highly endangered species by determining the overlap of seabird distribution and fishing activities (e.g. Hyrenbach & Dotson 2003; Cuthbert *et al.* 2005; Phillips *et al.* 2006), or the definition of appropriate marine protected areas for albatrosses and petrels (Awkerman *et al.* 2005; Terauds *et al.* 2006). For species with previous evidence of being affected by fisheries, the use of seabird locations determined by remote sensors, such as satellite transmitters or geolocators, and the simultaneous analysis of the distribution of

the fishing fleet is of increasing conservation relevance (Phillips *et al.* 2008). By using this approach a range of conservation measures could be delineated by focusing on particular fleets interacting with the species, establishing responsibilities to particular countries, and the establishment of spatial or temporal closures to reduce bycatch rates. In this study we tracked an endangered species the Spectacled Petrel *Procellaria conspicillata* on their wintering grounds in the southwestern Atlantic Ocean and we describe their habitat use in relation to oceanographic variables and distribution of the key fishing fleets.

The Spectacled Petrel was considered a subspecies of the White-chinned Petrel *Procellaria aequinoctialis* until recently (Ryan 1998). It is endemic to Inaccessible Island, in the Tristan da Cunha Archipelago, with a global breeding population of 9,000 pairs (Ryan *et al.* 2006), and is currently identified as 'vulnerable' to extinction (BirdLife International 2008). The species probably declined severely during 19th and early 20th century due to effects of feral pigs (Ryan 1998; Ryan *et al.* 2006). Population models indicate that the population is at risk of extinction primarily due to incidental capture in longline fisheries (Ryan *et al.* 2006). Demographic parameters for the species are scarce, but as is typical of Procellariiformes, they are expected to be long-lived birds with delayed maturity, high fidelity to partners and breeding sites, high adult survival and low fecundity (Warham 1990).

The at sea distribution of Spectacled Petrels was initially described to be throughout the South Atlantic in a band from Africa to South America between 25 and 40°S (Enticott & O'Connell 1985). However, it appears to be a regular, but scarce visitor off the African coast, with only 30 birds recorded during 629 10-minute counts (Camphuysen & van der Meer 2000). In the southwestern (SW) Atlantic Ocean, it is recorded from the Brazilian state of Bahia (12°S, Lima *et al.* 2004) to Falkland (Malvinas) Islands (*c.* 52°S), but is rare south of 40°S (Imberti 2002). Large concentrations occur in southern Brazil, where up to 300 birds (about 1.5% of the entire species' population) have been recorded following a single vessel (Neves *et al.* 2006b). In this region it is the most common pelagic seabird around the year in both continuous and snapshot censuses, with a strong tendency to follow longline fishing vessels (Olmos 1997; Neves *et al.* 2006b). It has been suggested that the species occurs preferentially over warm (Olmos 1997) and deep waters (Olmos & Bugoni 2006).

In the SW Atlantic Ocean the coastal branch of the Falkland (Malvinas) Current flowing northward and transporting Sub-Antarctic waters (4-15ºC) reaches warm waters of the Brazilian Current flowing southward and transporting tropical waters >20ºC (Garcia 1998). The convergence and mixture between the tropical and Sub-Antarctic waters forms the Subtropical Convergence (Garcia 1998), a highly productive area that supports

important fish stocks and considerable numbers of top predators (Seeliger *et al.* 1998). The coastal areas over the continental shelf further benefit from mixing with the outflow of continental waters. The separation between Brazilian and Falkland (Malvinas) Currents varies seasonally, from 35.8 to 38.6ºS, forming a heterogeneous area between both currents up to 1,000 km wide with many eddies and fronts (Olson *et al.* 1988). Farther north up to 23ºS the penetration of relatively cold waters (14-17ºC) occurs frequently in winter, mainly on the shelf break (Campos *et al.* 1996), which has an important influence on the composition of the seabird community (Olmos 1997).

The domestic Brazilian pelagic longline fleet is based at southern and southeastern ports and operates mainly in this area during winter and early spring months, targeting tuna, Swordfish and sharks (Bugoni *et al.* 2008a, Chapter 9). Considerable numbers of albatrosses and petrels are incidentally captured by the fleet, mostly Black-browed *Thalassarche melanophris* and Atlantic Yellow-nosed *T. chlororhynchos* Albatrosses, White-chinned and Spectacled petrels (Neves *et al.* 2006a; Bugoni *et al.* 2008a, Chapter 9). Despite Spectacled Petrels frequently attending longline vessels in higher numbers than the White-chinned Petrel (Olmos 1997; Neves *et al.* 2006b) the latter is the petrel with higher bycatch rates in the area (Jiménez & Domingo 2007; Bugoni *et al.* 2008a; Chapter 9). The reasons for differential capture rates among these similar sized and closely related species are unclear. Nocturnal foraging (which is unusual among most Procellariiformes) has been suggested as an important factor determining the incidental capture of White-chinned Petrels in South Africa and Kerguelen (Barnes *et al.* 1997; Ryan & Boix-Hinzen 1998; Weimerskirch *et al.* 2000) and setting hooks at night has been postulated as an effective mitigation measure to reduce bycatch of most seabirds (Cherel *et al.* 1996; Barnes *et al.* 1997). In the SW Atlantic, longline setting frequently takes place at night and this practice has also been advocated as an effective measure for reducing incidental capture of albatrosses and most petrels (Neves *et al.* 2006a). The effectiveness of nocturnal setting for avoiding Spectacled Petrel bycatch remains to be determined. Seabird associations with fisheries could be both detrimental, by reducing prey species used by birds or incidental mortality, or beneficial in the short term by providing baits and discards otherwise unavailable for birds (Furness 2003). Whether Spectacled Petrels benefit by feeding extensively on fishery discards (Neves *et al.* 2006a) or are adversely affected by the bycatch mortality they experience in this fishery (Bugoni *et al.* 2008a, Chapter 9) remains unclear. Improving knowledge of the ecology and behaviour of both Spectacled and White-chinned Petrels is crucial for understanding the differences in bycatch rates of these species, as well as our ability to propose effective mitigation measures to aid their conservation.

Some attempts have been made to quantify the overlap between longline fisheries and tracked albatrosses and petrels in the South Atlantic (e.g. Cuthbert *et al.* 2005; Phillips

et al. 2006). However, data on fishing effort used for comparison is frequently temporally non-overlapping with bird tracking periods, and is often based on incomplete datasets (Tuck *et al.* 2003), or fishing effort is only available on large scales (for example data provided by the International Commission for the Conservation of Atlantic Tuna -ICCAT- is in quadrants of 5º x 5º). For the study of pelagic animal distributions and their associations with fishing effort and oceanographic features, more fine scale definition is required, while 10-300 km is useful for core and range estimations (Nicholls *et al.* 2005). In addition to the problems of using incomplete or large scale data, temporal incongruence among fishing and bird data causes severe limitations due to the inherent high dynamicity of pelagic fisheries (Hyrenbach & Dotson 2003; Bugoni *et al.* 2008a, Chapter 9). Ideally, such studies would rely on concurrent telemetry and fishing effort data with a fine spatio-temporal resolution (Hyrenbach & Dotson 2003). The SW Atlantic is the most important feeding area for Spectacled Petrels throughout the year, and also the most important longline fishing ground for the Brazilian fleet during winter months. There this vulnerable species faces its main current threat, making the SW Atlantic the key area for its conservation. In this study we compare the marine habitats used by wintering Spectacled Petrels with fishing effort in quadrants of 1º x 1º, thus providing a fine-scale comparison of habitat utilization by birds and fisheries, over the same time period. We additionally investigated relationships between the distribution of birds and oceanographic features such as sea surface temperature (SST), chlorophyll *a* and bathymetry; the behaviour of birds during night time and daytime periods as an indicator of foraging behaviour and potential interaction with the usual night time setting of longline hooks; and we investigate the segregation in wintering areas between the sister species Spectacled and White-chinned Petrels, by comparing with previous information on White-chinned Petrel in Phillips *et al.* (2006).

METHODS

Sampling birds and transmitter attachment

Spectacled Petrels scavenging for discards at a pelagic longline vessel in southern Brazil were trapped using a cast net. This technique was considered practical and safe for birds and was extensively used recently in Brazil, with over 500 albatrosses and petrels from 14 species captured (Bugoni *et al.* 2008b). After trapping, birds were kept in a shaded area, alone in a box lined with cardboard for keeping them dry. Captured birds were measured, ringed with metal bands, checked for plumage moult which indicated if birds were first year juveniles (no flight feather or body feather moult) or older birds (signs of moult evident), and 1 mL of blood collected. Molecular sexing was carried out based on DNA extraction from blood and PCR amplification of CHD genes (Fridolfsson & Ellegren 1999), but the pair of primers used were 2550F (Fridolfsson & Ellegren 1999) and 2757R (R. Griffiths, unpublished).

Satellite transmitters were attached to back feathers using waterproof Tesa® tape 4651 (Wilson *et al.* 1997). One 30 g battery powered Platform Transmitter Terminal (PTT) from Microwave Telemetry was deployed in August 2006 and four 20 g battery PTTs (North Star Science and Technology) were deployed in August 2007. They correspond to 1.6-2.3% of petrels' body mass (1277.8 g, n = 20, Ryan 1998) and were configured to transmit continuously because we were interested in gathering as much data as possible on a fine spatial and temporal scale to allow determination of day and night time movements and association with fishing vessels. Egg laying at Inaccessible Island occurs around 20 October (Ryan 1998), thus all birds were in their wintering period when PTTs were deployed off Brazil in August. By attaching transmitters to wintering birds we could not perform a controlled assessment of effects of PTTs on birds, but potential detrimental effects were minimized by limiting devices to < 3% of Spectacled Petrels' body mass, using waterproof tape instead of harness, and by tracking birds for short periods, following best practices for satellite tracking of Procellariiformes recommended by Phillips *et al.* (2003).

Satellite performance

Locations of birds were monitored using the ARGOS system, which provided data on latitude, longitude, day, GMT time and accuracy of each location. Data on tracked birds provided by ARGOS were processed using the software DSDCODE, Version 3.D (Geotrak Inc.). When tracking animals which spend large periods underwater or when the period of transmission is not continuous, a much larger proportion of auxiliary locations (A, B and Z) is obtained, for example 66% in Grey seals *Halichoerus grypus* (Austin *et al.* 2003). In these cases some filtering methods are commonly used to save less accurate data. Here we carried out a conservative approach and removed all Z, A and B locations. These auxiliary locations accounted for only 13.5% of all gathered locations (n = 7400). The remaining set of data was subsequently filtered using an iterative method (McConnell *et al.* 1992) to remove unrealistic locations assuming a maximum flight speed of 90 km h^{-1} (Phillips *et al.* 2006). During this procedure we tried to remove less accurate data first (LC 0), and the lowest possible number of points, which removed an additional 272 points (3.7% of the total). Time interval between valid locations varied between PTTs, from 21 to 44 min on average. With larger battery, the PTT deployed in 2006 resulted in 3167 valid locations during 49 tracked days, while the four PTTs deployed in 2007 resulted in a mean of 741 ± 146 locations (range 577 to 905) and tracked petrels during 17 to 20 days each (Table 8.1). In total, 6132 bird locations were used for the analysis.

Marine habitat utilization

The nonparametric fixed kernel density estimator (Wood *et al.* 2000) was used to determine the 80, 60, 40 and 20% density contour areas (i.e. the estimated home range), which are a weighted frequency of locations within the specified radius (Wood *et*

al. 2000). Densities contours corresponding to kernels were calculated for locations in a Lambert Equal-Area Azimuthal projection centred on the South Pole. Arcview GIS 9 was used for calculations with Hawth's Analysis Tools for ARGIS (Beyer 2004), setting cell size at 5 km, and parameter smoothing factor (*h*) at 40 km. Locations from radio and satellite tracking are typically not serially independent, but independence is not a requirement for kernel analysis (De Solla *et al.* 1999; Nicholls *et al.* 2005). Collecting more frequent locations increases the autocorrelation between points, but large sample size has been shown to be more important than independence for home range analysis (Seaman *et al.* 1999 and references therein).

Oceanographic features

SST in degrees Celsius and chlorophyll *a* concentrations in mg m^{-3} were obtained from Aqua MODIS mapped products at 4-km cell resolution from National Atmospheric and Space Administration (NASA) (http://oceancolor.gsfc.nasa.gov/) through the mirror at University of São Paulo (http://orion.io.usp.br/los/). Bathymetry was obtained from the General Bathymetric Chart of the Ocean (GEBCO) at http://www.bodc.ac.uk/data/online_delivery/gebco/.

Bathymetric domains were defined as coastal (< 200 m), continental shelf (200 to 1000 m), shelf break (1000 to 3000 m) and pelagic (> 3000 m). SSTs were classified according to the definition of water masses expected to occur in the Subtropical Convergence, following Garcia (1998): Tropical water (> 20ºC), SubAntarctic waters (4-10ºC) and Subtropical waters, resulted from the mixture of both (10-20ºC). In fact the range of SST of the SubAntarctic waters from the Falkland (Mavinas) Current is from 4 to 15ºC and the salinity is used with temperature to define water masses, but because we did not obtain salinity data and to avoid overlap between temperature ranges, waters were defined arbitrarily. Chlorophyll concentrations were divided in four classes after Hyrenbach *et al.* (2002): oligotrophic (concentration below 0.1 mg m^{-3}), mesotrophic (0.1 to 0.3 mg m^{-3}), eutrophic (0.3 to 1 mg m^{-3}), and enriched waters (> 1 mg m^{-3}).

Night and daytime movements

Each satellite location was classified as nocturnal or diurnal using the nautical dawn and dusk definitions, i.e. the time when the center of the sun is 12º below the horizon, using the calculation provided in http://www.srrb.noaa.gov/highlights/sunrise/sunrise.html) which has accuracy of ± 1 minute. Night and daytime travelled distances were calculated using the great circle distance and speeds of tracked petrels calculated for each night or day period based on the first and last locations. For testing the differences between velocity and distance travelled during night and day periods we used the Mann-Whitney test, differences between birds were compared with Kruskal-Wallis test, and marine habitat preferences according to bathymetry, productivity and SST by χ^2 test considering

the number of locations in each domain, without arbitrarily defining the availability of habitats (Minitab®, v. 15).

Fishing effort

Pelagic longline fishing effort for the domestic fleet was obtained through weekly visits to Itajaí and Santos harbours by Projeto Albatroz Team and in Itajaí by daily visits by the Grupo de Estudos Pesqueiros, Universidade do Vale do Itajaí (GEP-UNIVALI). Logbooks were obtained from captains and complemented with interviews, and contained information on fishing grounds (1° x 1° lat-long resolution) and number of hooks deployed per day or for the whole trip. Data from different sources were compared looking for the largest coverage possible and to avoid overlap, restricted to the tracking period of birds. The 2007 Brazilian pelagic longline fleet in ports located in south and southeastern Brazil comprised 37 vessels and the coverage by the sampling described above was over 70% of the total for this fleet. Comparison between bird habitat utilization and fishing grounds was limited to 2007, when representative data on birds and fishing effort were obtained. Data for the leased fleet effort during 2006 was available through the Report to the Regional Fishing Organization ICCAT, but was not used here because the main effort of this fleet occurred in areas northward or west of the area of interest for the birds (see also Bugoni *et al.* 2008a, Chapter 9 for fleet dynamics). In addition, from a total of 5.1 million hooks deployed by the leased fleet during 2006, only 221,327 hooks (4.3%) were deployed from August to October 2006 and between 25-40ºS and 40-55ºW, corresponding to the periods and areas of tracked birds. Data from the leased fleet during 2007 were not available when preparing this paper, but it is expected to represent a minor proportion of the longline fishing effort in the area. Additionally, tracking data were not compared with the bottom longline and the 'Itaipava-type' hook-and-line fishery described by Bugoni *et al.* (2008c). Both could operate in the area, but the former now represents a small proportion of the late 1990s fleet (Neves *et al.* 2007), and the latter, which captures Procellariiformes including the Spectacled Petrel, operates in southern Brazil only during warm months (Bugoni *et al.* 2008c). Thus, these fleets were not overlapping with the tracking period and areas reported here or were considered of minor importance. Furthermore, because core areas used by birds were mostly within the Brazilian Exclusive Economic Zone (see Results), fishing effort from other countries was not considered.

RESULTS

General movement characteristics and kernel density

All Spectacled Petrels tracked in 2007 were males (based on molecular sexing), and were over one year-old, because they were moulting body feathers (dorsum, ventral and head) when PTTs were deployed in August. The PTT deployed in 2006 worked for 1176 h or 156% over the expected lifetime of 750 h. PTTs deployed in 2007 provided uplinks for

similar periods (17 to 20 days) (Table 8.1), suggesting that all PTTs stopped transmitting due to battery exhaustion.

The bird tagged in 2006 and three out of four birds tagged in 2007 foraged predominantly in waters of the Brazilian EEZ in southern Brazil. The distribution of these four birds formed a band oriented NE-SW, corresponding to the shelf break, from 25 to 33ºS (Fig. 8.1). The bird tagged in August 2006 at 28ºS over the continental shelf stayed over the shelf break for about one month during which period it had a trip of one week to offshore waters, 500 km east to the shelf break. From 23 September it headed towards Tristan da Cunha/Inaccessible Islands in the mid-Atlantic where the species breeds. When close to the islands the bird moved northwest, crossing close to the Rio Grande Plateau where the signal was lost.

Three birds tagged in 2007 in the southern portion (33-34ºS) over relatively cold waters moved north-eastward two days after tagging. The other bird (75850) also moved northeastward, but over deep waters off the Brazilian EEZ, stayed for a couple of days over the Rio Grande Plateau, after heading southwestern up to 41ºS and northwestern again (Fig. 8.1).

Core home range areas defined by kernel densities 20% and 40% demonstrated intense use of the shelf break between 25 and 31ºS (Fig. 8.2), with 40% kernel areas between 16,753 to 116,325 km^2 (Table 8.1). The largest area was of the bird which did not stay over the shelf break as all the others, but wandered considerably over offshore waters. The kernel area generated by all 2006 and 2007 locations was 34,007 km^2 at 20% density and 596,545 km^2 at 80% and included some small offshore patches (Fig. 8.2).

Diel travelled distances and speed

Mean speed and distances travelled varied between birds (speed: Kruskal-Wallis H = 31.9, df = 4, $P < 0.001$; distances: H = 40.5, df = 4, $P < 0.001$), but there was no difference between day and night in speed or distance travelled (speed: Mann-Whitney W = 12391.5, $P = 0.7$; distance - W = 13306, $P = 0.1$). Every bird moved from 98 to 216 km on average during daylight hours, with average speed varying from 9 to 21 km h^{-1} (Table 8.3). At night the average distance varied from 104 to 232 km, and speed varied from 14 to 24 km h^{-1}. Results were similar by analysing each bird individually, with no difference between speed or distance travelled during day and night, with the exception of the bird tracked in 2006 which travelled further during daylight (Table 8.3). Thus, in spite of individual differences, the Spectacled Petrel has similar activity levels during night and day. Furthermore, distances and speed during daytime were correlated with distances and speed during the subsequent night in four out of five birds (correlations coefficients from r = 0.27 to r = 0.66, n = 15 to 44, all $P < 0.05$). Thus, the species

spent several days commuting between foraging grounds, in which periods they sustain high speed and travel farther, and periods of low mobility, when they travel short distances and more slowly. The latter case is expected to represent periods of foraging activities intercalated with periods of resting on the water.

Oceanographic domains

The four bathymetric domains were not used evenly by the birds, which clearly preferred the shelf break and offshore deep waters (Figs. 8.1 & 8.2) rather than the continental shelf (χ^2 = 3747.2, df = 3, P < 0.0001, Fig. 8.4). Fifty-two percent of locations were over water > 3000 m, 34% between 1000 and 3000 m and only 0.6% over the continental shelf < 200 m. Average water depth at kernel 20% was 1043 ± 794 m and at 40% was 2250 ± 1353 m (Table 8.2).

Birds stayed over warm Subtropical and Tropical waters (mean SST 22.05 ± 0.31°C and 20.95 ± 1.25°C in 2006 and 2007, respectively). Only 1% of locations were over cold waters of the Falkland (Malvinas) Current (Fig. 8.3), which were present in the area over the continental shelf and southward (Fig. 8.4). Birds sampled in 2007 were tagged over cold waters, but moved northward or offshore soon after PTT deployment. There was a clear preference for subtropical and, to a lesser extent, tropical waters (χ^2 = 3302.5, df = 2, P < 0.0001 n = 6073 locations, Fig. 8.3).

Mesotrophic waters (chlorophyll *a* density between 0.1 and 0.3 mg m^{-3}) represented 81% of all locations, with minor proportion of oligotrophic and eutrophic waters (8 and 12%, respectively, Fig. 8.3). Eutrophic and even enriched waters were present at the area during the tracking period near the coast and southward, but were used less than expected (χ^2 = 9912.4, df = 3, P < 0.0001, n = 5887 locations). The 20% kernel density areas used by birds occurred over water with chlorophyll density between 0.14 and 0.95 mg m^{-3}, average in 2006 of 0.301 ± 0.71 mg m^{-3}, and in 2007 of 0.281 ± 0.65 mg m^{-3} (Table 8.3).

Overlap with longline fishery

The pelagic longline fishery effort in August 2007 was concentrated along the shelf break off southern Brazil, in a SW-NE oriented band, similar to the distribution of satellite locations and the kernel distributions of birds (Fig. 8.6). The fishing effort of the domestic longline fleet was concentrated between 26º and 32ºS, where 77.5% of hooks were deployed during the tracking period (n = 787,834 hooks sampled). The areas with the highest density utilization by birds (kernel density < 40%) were those with highest fishing effort. Considering all 2007 locations, 47% were inside quadrants where pelagic longline hooks were deployed, even considering one bird that spent all time far offshore in an area where fishing data was not available. Furthermore, the number of locations

was higher in quadrants with highest number of hooks deployed (Spearman rank correlation r_s = 0.47, n = 23, *P* = 0.027), demonstrating that birds stayed longer in areas of intense pelagic longline fishery. It is also noticeable that although birds were tagged in the southern areas where the fishing effort was low, they subsequently moved and stayed in areas with intense fishing activities to the north. One bird had no association with this fleet by moving over a vast area over international waters where the fleet was not operating during this time. The density distribution for this bird was not concentrated in a particular area (Fig. 8.2), which suggests that if this individual attended some fishing vessels it did so only over short periods. The other birds could be commuting between vessels operating nearby, but to investigate the association of each bird with a particular vessel we should require higher resolution data, with birds and vessels tracked by GPS satellite transmitters.

DISCUSSION

Foraging areas of wintering birds

The distribution of Spectacled Petrels at sea is poorly known, and this is the first study to describe the foraging areas of the species based on satellite telemetry. Most studies using satellite technologies to track seabirds deploy PTTs in colonies and are restricted to breeding periods, just after chick fledging or when the adults fail to breed (but see Hyrenbach & Dotson 2003; Suryan *et al.* 2007). During wintering periods seabirds are no longer central place-foragers and they are able to explore the marine environment more widely, changing foraging grounds if convenient or being associated with particular oceanographic areas or fisheries (Bugoni *et al.* 2005; Phillips *et al.* 2008). The long displacement of three birds, just after tagging, from 34ºS to 29-30ºS is explained by birds taking advantage of polar fronts crossing the area, which typically have strong S-SW winds and are common during winter and spring. During satellite deployment calm wind conditions predominated and there were just a few Spectacled Petrels over cold waters. Albatrosses and petrels commonly take advantage of tail and cross winds (Jouventin & Weimerskirch 1990; Falk & Møller 1995), since the costs of displacement are reduced with high-speed winds (Furness & Bryant 1996).

The bird tracked in 2006 headed towards Tristan da Cunha Islands at the end of tracking (Fig. 8.1) after a long period in the SW Atlantic, and thus was probably an adult bird or a prospecting immature. Birds tracked in 2007 were in late moult stage, probably preparing for breeding, as Spectacled Petrels are expected to replace wing and tail feathers before body feathers (Warham 1990).

All birds except one used the shelf break area extensively, as demonstrated by the kernel analysis (Fig. 8.2), with occasional incursion to offshore deep waters, or when heading for breeding grounds. However, these movements were rapid, with no particular area in

international waters used preferentially. It has been demonstrated that the Rio Grande Plateau, a shallow area around 30ºS-35ºW (Fig. 8.1) is a place of large flocks of Spectacled Petrels throughout the year, and where they meet the Brazilian longline fleet during summer (Bugoni *et al.* 2006), and this area was clearly of interest to tracked birds.

Diel foraging activities

Spectacled Petrels travelled similar distances and at similar speeds during day and night. These results contrast with other albatrosses and petrels for which day and night differences in these parameters were found. Some studies show albatrosses and petrels flying further and faster at night (Klomp & Schultz 2000; Nel *et al.* 2000; Hyrenbach *et al.* 2002) or landing more frequently during the day (Fernández & Anderson 2000). Catry *et al.* (2004) found that Gray-headed Albatross *Thalassarche chrysostoma* spent 94% of the night on the water, apparently sleeping as they consumed only 26% of their prey during night. Nel *et al.* (2000) assumed that the speed of < 10 km h^{-1} recorded for Gray-headed Albatross during daylight hours was indicative of concentrated foraging. For the White-chinned Petrel, Phillips *et al.* (2006) found high levels of nocturnal activity, measured as speed and distance travelled, but high activity in this case refers to birds performing long flights instead of foraging. In the present study correlations between day and night speeds and distances travelled in subsequent periods suggest that Spectacled Petrel activity might be divided in two different types, representing different foraging strategies: periods of one or more days performing long and fast flights apparently commuting between foraging areas or between breeding grounds and foraging grounds; and periods when they stay relatively stationary over productive foraging grounds, or associated with vessels. However, this pattern seems to be independent of day or night. Weimerskirch & Guionnet (2002) determined that four albatross species spent 55-65% of their time in flight and during the night they spent most of their time sitting on the water, while Catry *et al.* (2004) using stomach temperature loggers, satellite tracking and wet-dry activity loggers simultaneously demonstrate that when sitting on the water and under complete darkness, Gray-headed Albatrosses are able to ingest considerable amounts of food. Pinaud & Weimerskirch (2007) also found long stationary periods during nigh when analysing seven satellite tracked Procellariiformes including the White-chinned Petrel. From these results of diel activity in a range of seabirds, it is not clear if lower activities at night, measured as speed or absolute travelled distance, represent foraging behaviour, birds resting on the water, or even opportunistic feeding during resting periods at night. Procellariiformes, and Spectacled Petrels in particular, are frequently observed roosting at sea in flocks after feeding to satiation, or in early morning before the longline hauling starts (L. Bugoni, pers. obs.). In addition, slow speed and short distances covered by birds following fishing vessels is also expected during longline hauling, when vessel speed is 5-10 km h^{-1}. When birds arrive in foraging

grounds or find a profitable feeding area, they appear to move short distances, and show lower mean speeds, adjusting the scale of movements and speed according to the species and environmental characteristics (Weimerskirch *et al.* 1997; Pinaud & Weimerskirch 2005, 2007). The distinction between feeding and resting could not be made based on satellite uplinks alone. For a better understanding of diel foraging activities a complementary approach is necessary by using simultaneously satellite transmitters and stomach temperature loggers (e.g. Catry *et al.* 2004), a procedure only feasible in colonies because the temperature logger needs to be recovered for downloading the data.

Furthermore, some individuals appear to behave differently, by covering larger distances away from longline fishing grounds, such as the bird 75850 (Fig. 8.1). Whether this bird was commuting from a vessel to another or feeding on prey naturally available is unknown, but individual feeding preferences, with some individuals more prone to scavenge for fishing discards or attend different foraging grounds is a well described phenomenon in seabirds (Freeman *et al.* 2001; Phillips *et al.* 2005b) and could have occurred in this case.

Oceanography and segregation with White-chinned Petrel

Spectacled Petrels prefer warm, offshore and mesotrophic/oligotrophic waters of the SW Atlantic Ocean. The cold waters of the Falkland (Malvinas) Current are more evident over the continental shelf and north up to around 30ºS at southern Brazil where high productivity also takes place (Figs. 8.4 & 8.5). Coastal cold and productive waters are used extensively by wintering White-chinned Petrels (Phillips *et al.* 2006), but were clearly avoided by Spectacled Petrels, as they were tagged in the south but moved quickly north, and high density use of the region by both species does not overlap extensively. The density distribution in 2006 and 2007 also demonstrates a clear preference for warm and mesotrophic waters found offshore and further north. The segregation pattern demonstrated here is in accordance with low numbers of Spectacled Petrels among stranded birds on the coastline, while the White-chinned Petrel is more common (Colabuono & Vooren 2006), as well as with the high abundance of Spectacled Petrels in southeastern Brazil (Olmos 1997) and their association with deep waters as demonstrated by at sea censuses and canonical analysis (Neves *et al.* 2006b). That these closely related species show remarkably different at sea distributions and association with SST, bathymetry and productivity, gives additional support for the distinctiveness of taxa and the specific status proposed by Ryan (1998) for the Spectacled Petrel based on morphometric data, plumage characteristics, vocalizations and playback responses. Furthermore, while both are associated with pelagic longline fishery occurring over the shelf break, White-chinned Petrel is expected to interact more extensively with a range of other fisheries taking place over the continental shelf, such as bottom trawling, gillnet,

and bottom longline. Environmental heterogeneity such as that found in the Subtropical Convergence area in the SW Atlantic Ocean, coupled with habitat selection provide a basis for species coexistence, as suggested for closely-related greater albatrosses (Waugh & Weimerskirch 2003).

White-chinned Petrels breeding in South Georgia travelled at mean flight speeds of 29.9 and 36.6 km h^{-1}, for males and females respectively (Berrow *et al.* 2000). These values are higher than Spectacled Petrel mean speeds in Brazil, which varied from 9 to 24 km h^{-1} (Table 8.2). However, during breeding White-chinned Petrels commute mainly to the Patagonian shelf, far from South Georgia where they breed. Thus, foraging trips are typically composed by one period when they fly fast going to and coming from foraging grounds, and a period with slow speed in foraging grounds. Moreover, White-chinned Petrels' mean flight speed of males (29.9 km h^{-1}) was lower than that of females (36.6 km h^{-1}) and similar between night and day during incubation (33-34 km h^{-1} sexes pooled), but lower at night (25 km h^{-1}) during chick-rearing (Berrow *et al.* 2000).

It has been demonstrated that components of the guild of highly mobile generalist avian predators from South Goergia overlap in foraging areas and have similar diet, except the Light-mantled Sooty Albatross *Phoebetria palpebrata* and the White-chinned Petrel (Phillips *et al.* 2005a, 2008). Coexistence is facilitated to a large extent by spatial segregation in foraging areas, and inter-specific intensity of competition is reduced by differences in behaviour (Phillips *et al.* 2008).

Interactions with fisheries

Despite being trapped behind longline vessels we believe that tracked birds are representative of the population because other banded birds remained for short periods attending the vessel displaced hundreds km just after taggind. In addition Spectacled petrels have high flying ability and could easily commut between vessels and 'natural' foraging sources if available.

The Brazilian pelagic longline fishing effort targeting Swordfish *Xiphias gladius*, Tunas *Thunnus albacares*, *T. alalunga* and *T. obesus*, and Sharks (mainly Blue Shark *Prionace glauca*) has varied over recent years, with an increase in the proportion of the domestic fleet and an effort of 9 million hooks in 2006 (Bugoni *et al.* 2008a, Chapter 9). This fishery and the similar Uruguayan pelagic fleet (Jiménez & Domingo 2007) capture Spectacled Petrels mainly during winter months, when the fleet concentrates efforts between 20-50ºS. In addition, Spectacled Petrels are captured in a range of hook-and-line fisheries during summer months (Bugoni *et al.* 2008c) with the highest capture rate per species in the handlining fishery targeting tuna, but also in the slow trolling targeting Bigeye Tuna. The surface longline for Dolphinfish *Coryphaena hippurus* has a high

potential for interaction with the species during summer (Bugoni *et al.* 2008c). Moreover, birds are also expected to spend considerable time in Uruguayan and Argentinean waters during the summer, when their preferred warm waters spread south, as well as in international waters, where longline fleets from these and other countries such as Japan, Taiwan, Korea, and Spain deploy considerable numbers of hooks (Tuck *et al.* 2003).

The Spectacled Petrel population was estimated to be at least 20,000 individuals, continuing an apparent recovery from a very small population in early 20th century (Ryan *et al.* 2006). Neves *et al.* (2006a) demonstrated that Tristan da Cunha and Gough Islands are the main breeding grounds for albatross and petrel species found in the SW Atlantic, with 11 out of the 22 species, and including endemic and threatened species, such as Tristan Albatross *Diomedea dabbenena*, Atlantic Yellow-nosed Albatross *Thalassarche chrlorhynchos,* and Spectacled Petrel. The longline fishery is the common cause for concern for the conservation of these species (Cuthbert *et al.* 2003b; 2005). A crucial step to the conservation of albatross and petrel populations affected by fisheries is describing the population ranges, assessing the susceptibility of birds and highlighting national responsibilities (Hyrenbach & Dotson 2003). In this way, the current study determined the distribution of Spectacled Petrels and demonstrated the great overlap with the Brazilian pelagic longline fleet during winter.

Similar to our results, Terauds *et al.* (2006) found high overlap between the distributions of satellite-tracked Black-browed Albatrosses and trawler fishing around Macquarie Island. On the other hand, little overlap between longline fishing and satellite tracked Black-footed *Phoebastria nigripes* and Laysan *P. immutabilis* Albatrosses was found in the North Pacific Ocean (Hyrenbach *et al.* 2002), a discrepancy attributed to different sampling periods. In investigations of seabird distributions and overlap with fisheries the temporal scale and the spatial fine-scale resolution is essential.

Phillips *et al.* (2006) found higher distances and speed of White-chinned Petrels at night, suggested to represent low foraging activities, which contrast with Spectacled Petrel, which has similar speed and travelled distance by day and night. The role of this behavioural difference between White-chinned and Spectacled Petrel is unclear, as is how it relates to risk of incidental capture on longlines. A possible explanation for the higher mortality of White-chinned Petrel in comparison with Spectacled Petrel in pelagic longline fishery could be differences in age, as the former are mostly juveniles and the later are mostly subadults and adults (Chapter 6).

Conservation implications

Recently, the bycatch of seabirds has been addressed by the Brazilian government and non-governmental organizations, with the launch of the National Plan of Action for the

Conservation of Albatrosses and Petrels – NPOA/Brazil in 2006 (Neves *et al.* 2006a); the signature of the Agreement for the Conservation of Albatrosses and Petrels – ACAP in 2001, not yet ratified by the Brazilian government; the testing of mitigation measures such as bird scaring lines and blue-dyed baits (Neves *et al.* 2008), which should be compulsory soon; the satellite tracking of each longline vessel of the leased fleet, and the domestic fleets being implemented since 2006; and the proposed coverage of the fleet by onboard observers, currently under consideration by the Brazilian Environmental Agency. These actions are significant progress towards the mitigation of the global problem of incidental capture of albatrosses and petrels in fisheries. However, no mitigation measure applied to fishery is expected to be effective in Brazil without strong enforcement (Bugoni *et al.* 2008c). The importance of Brazilian waters and the implications of the national longline fleet for the conservation of Spectacled Petrel have been demonstrated in this study, which certainly extends to other jurisdictional countries and international fleets at different periods of the annual cycle of the species. Overall, Spectacled Petrels have been demonstrated to prefer relatively warm and oligotrophic waters over the shelf break and offshore, with great overlap with the pelagic longline fishery. Spectacled Petrel remains a poorly known species, vulnerable, strongly associated with fishing vessels, and with population concentrated in the SW Atlantic Ocean. Therefore, Brazil and other countries fishing in the area under the ICCAT Regional Fishery Organization jurisdiction have a critical role in the conservation of this species.

Table 8.1. *Procellaria conspicillata*. Summary of data from the five tracked Spectacled Petrels in southwestern Atlantic Ocean in 2006 and 2007.

Bird	Sex	Tracking period	Tracked days	Number of valid locations	Total distance travelled (km)	Overall mean speed (km h^{-1})	40% kernel area (km^2)
07783	Unknown	17 Aug – 04 Oct 2006	49	3167	17,891	15.3	75,406
75850	Male	01 Aug – 20 Aug 2007	20	905	10,124	22.6	116,325
75851	Male	01 Aug – 18 Aug 2007	18	814	7310	18.4	39,784
75852	Male	01 Aug – 17 Aug 2007	17	669	4501	12.0	17,645
75853	Male	06 Aug – 24 Aug 2007	19	577	5853	13.4	16,753

Table 8.2. *Procellaria conspicillata*. Mean ± 1 SD and range of water depth, sea surface temperature and chlorophyll *a* concentration within different kernel density distributions of Spectacled Petrels in the southwestern Atlantic Ocean.

Kernel density (%)	Kernel area (km^2) 2006 and 2007	Depth (m) 2006 and 2007	SST (°C) 2006	SST (°C) 2007	Chlorophyll *a* (mg m^{-3}) 2006	Chlorophyll *a* (mg m^{-3}) 2007
20	34,008	1043 ± 794 (153 – 2138)	22.05 ± 0.31 (21.40 – 22.60)	20.95 ± 1.25 (15.40 – 21.70)	0.301 ± 0.71 (0.14 – 0.83)	0.281 ± 0.65 (0.15 – 0.95)
40	105,194	2250 ± 1353 (121 – 4758)	20.79 ± 1.20 (17.60 – 22.60)	19.36 ± 2.71 (4.20 – 21.90)	0.273 ± 0.63 (0.12 – 2.50)	0.253 ± 0.53 (0.12 – 2.29)
60	254,733	3229 ± 1493 (105 – 5165)	18.84 ± 2.91 (6.90 – 22.60)	18.10 ± 3.03 (3.20 – 22.00)	0.253 ± 0.57 (0.08 – 2.56)	0.203 ± 0.53 (0.07 – 2.55)
80	596,545	3701 ± 1324 (93 – 5186)	17.76 ± 2.87 (6.80 – 22.60)	17.21 ± 3.16 (2.59 – 22.10)	0.243 ± 0.55 (0.07 – 3.70)	0.176 ± 0.54 (0.07 – 3.70)

Table 8.3. *Procellaria conspicillata*. Activity levels of Spectacled Petrels in the southwestern Atlantic Ocean in 2006 and 2007 during daylight and nightime periods measured by mean distanced travelled and speed ± 1 SD.

Bird	Distance travelled day (km)	Distance travelled at night (km)	Mean speed day (km h^{-1})	Mean speed at night (km h^{-1})
07783[a]	184.8 ± 174.3	104.0 ± 95.8*	18.0 ± 13.8	13.8 ± 10.8[ns]
75850	227.3 ± 84.6	232.1 ± 100.9[ns]	21.2 ± 7.9	23.5 ± 9.7[ns]
75851	215.8 ± 113.0	166.4 ± 83.8[ns]	19.2 ± 9.0	17.2 ± 7.5[ns]
75852	97.9 ± 56.2	132.0 ± 88.1[ns]	9.0 ± 4.5	14.4 ± 7.5[ns]
75853	125.9 ± 122.6	129.0 ± 83.7[ns]	12.7 ± 12.3	14.7 ± 7.7[ns]

[a] During last ten days it headed towards Inaccessible Island where they breed, travelling a mean of 426.1 ± 250 km during the day (speed 38.4 ± 16.3 km h^{-1}) and 168.2 ± 131.7 km during the night (speed 22.6 ± 14.4 km h^{-1}).

* statistically significant at $P < 0.05$

[ns] differences not statistically significant.

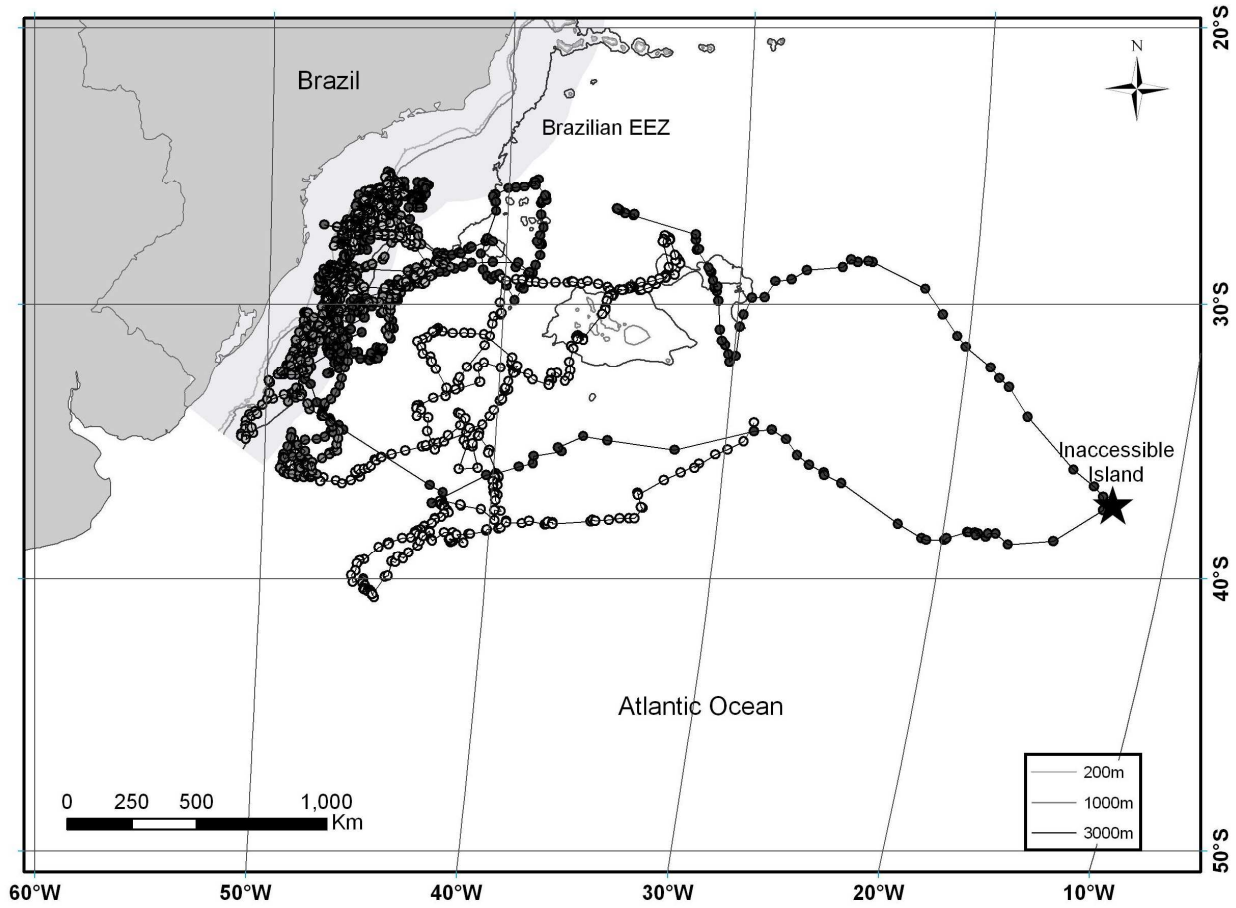

Figure 8.1. *Procellaria conspicillata*. Distribution of satellite locations (n = 6132) and movements of five Spectacled Petrels tracked in winter 2006 and 2007 in relation to bathymetry.

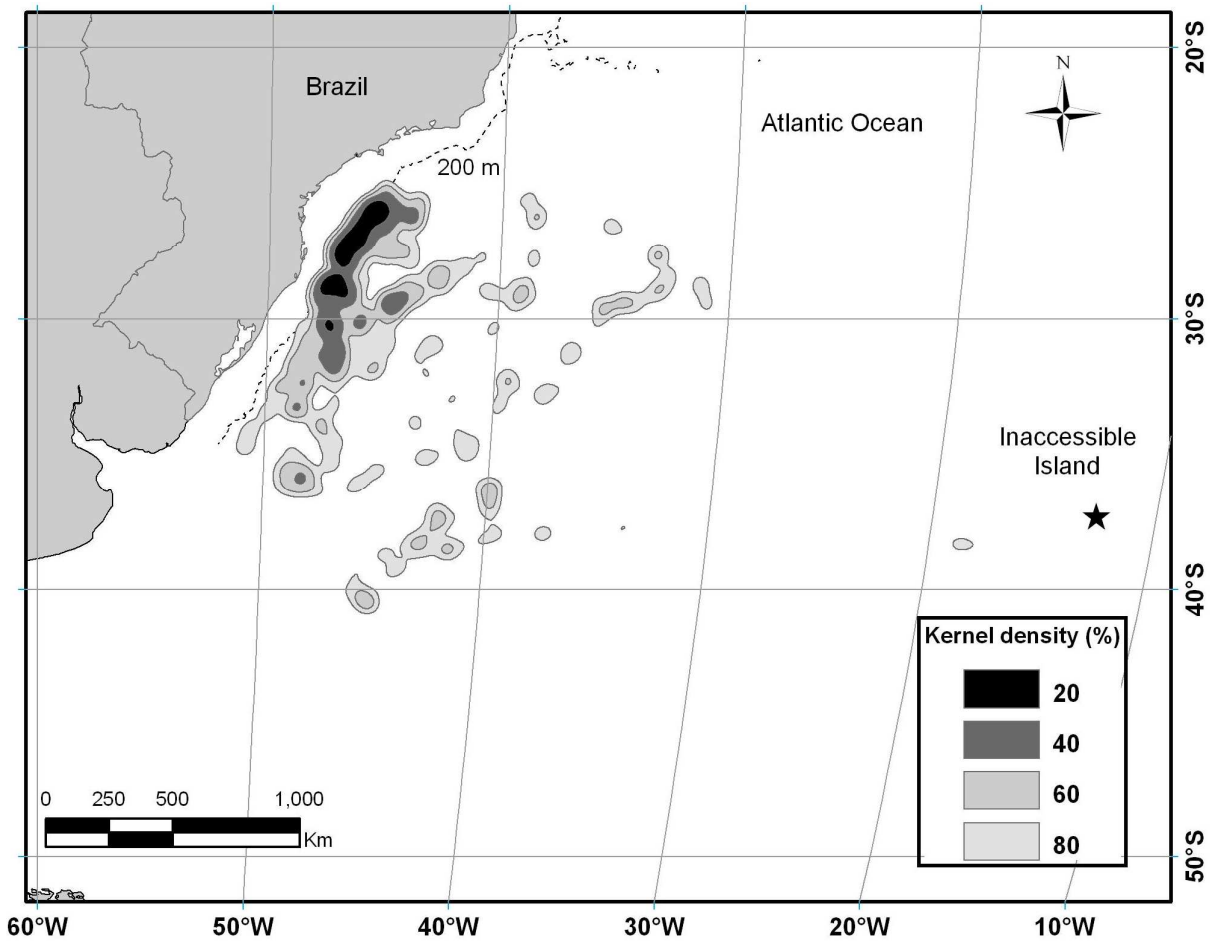

Figure 8.2. *Procellaria conspicillata*. Kernel density distribution of five Spectacled Petrels tracked during wintering period in 2006 and 2007.

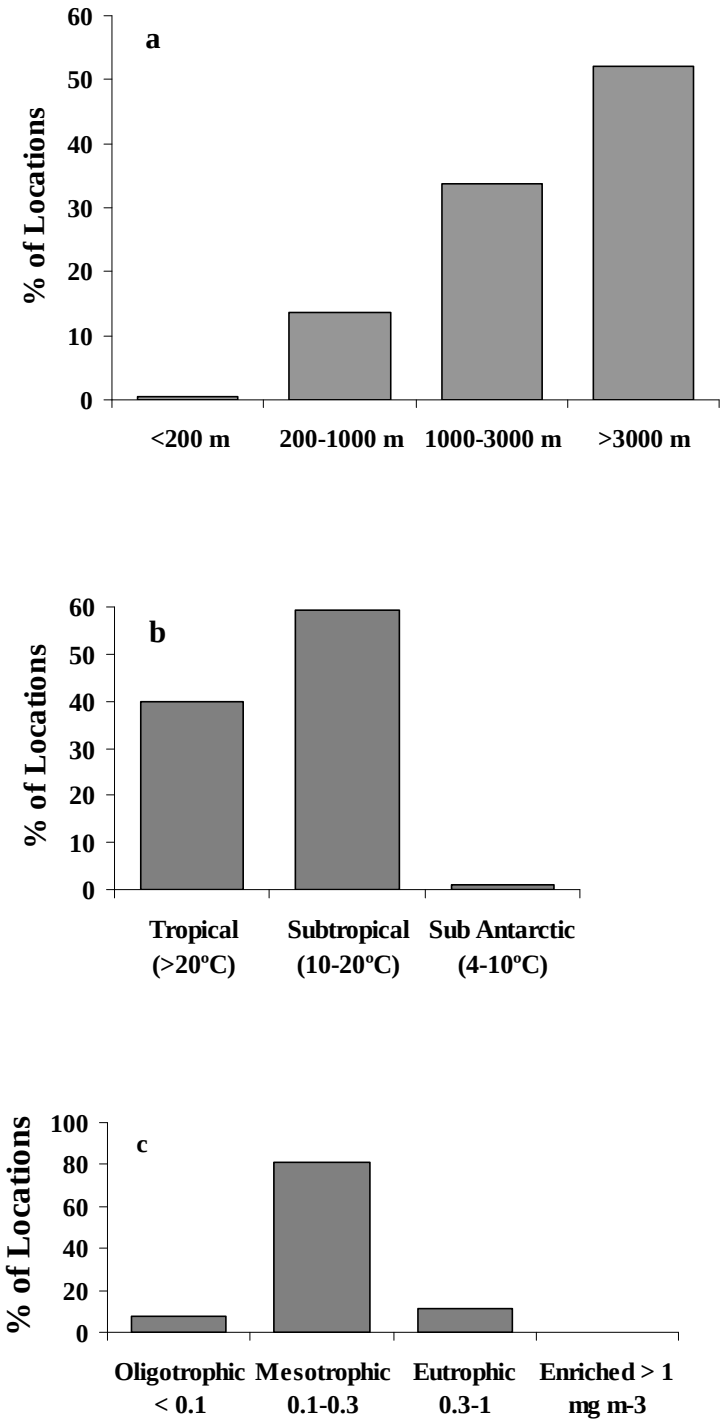

Figure 8.3. *Procellaria conspicillata*. Frequency of locations (%) of Spectacled Petrels within each bathymetric domains (a), SST (b) and chlorophyll *a* concentration (c).

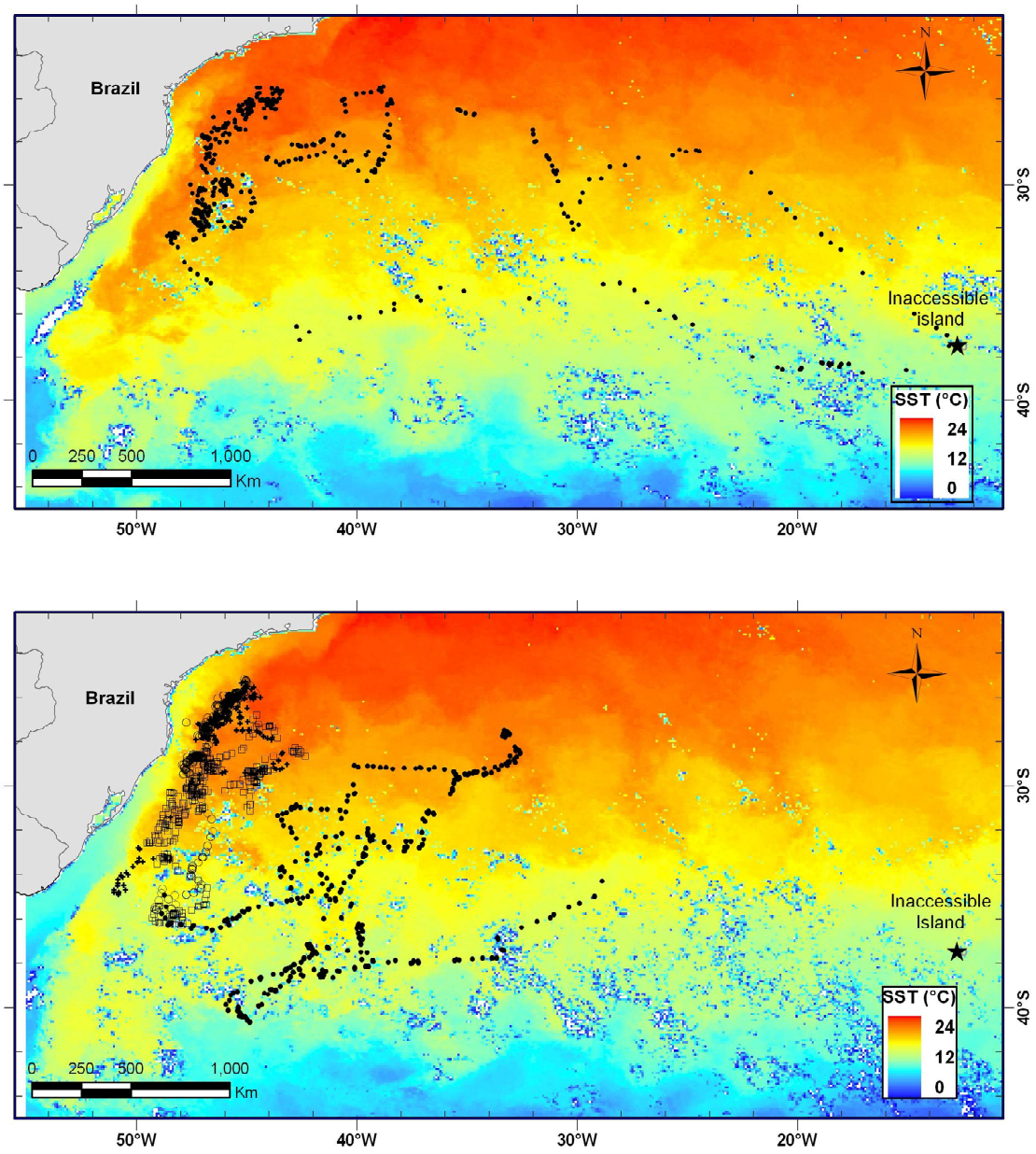

Figure 8.4. *Procellaria conspicillata*. Habitat preference of Spectacled Petrels tracked during wintering period in 2006 (above) and 2007 (below) in relation to water masses defined according to SST. Individual birds are represented with different symbols as in Figure 8.5.

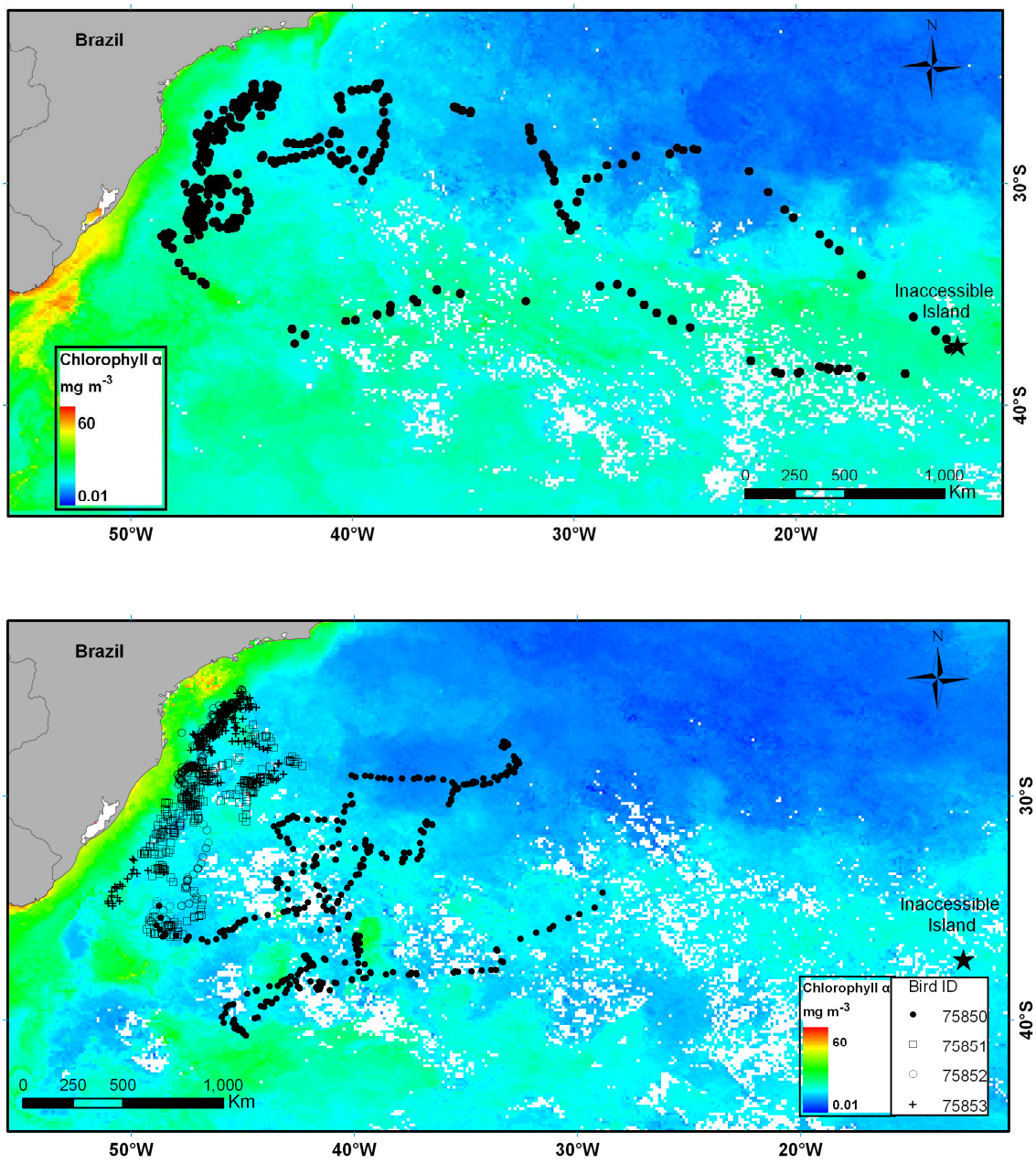

Figure 8.5. *Procellaria conspicillata*. Habitat preference of Spectacled Petrels tracked during wintering period in 2006 (above) and 2007 (below) in relation to chlorophyll *a* concentration. Individual birds are represented with different symbols.

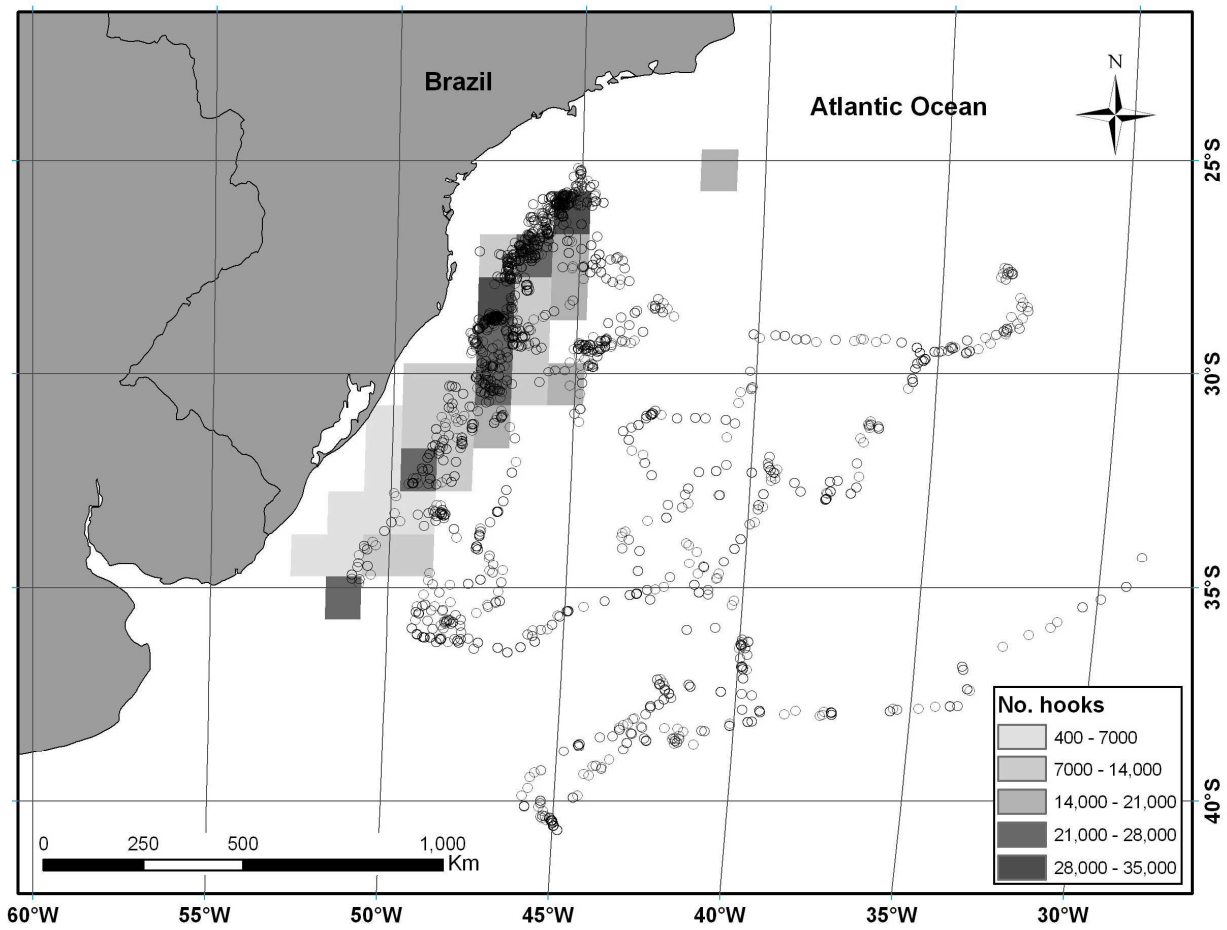

Figure 8.6. *Procellaria conspicillata*. Distribution of fishing effort in number of hooks deployed by the Brazilian domestic longline fleet during the 2007 tracking period of Spectacled Petrels.

Chapter 9

SEABIRD BYCATCH IN THE BRAZILIAN PELAGIC LONGLINE FISHERY AND A REVIEW OF CAPTURE RATES IN THE SOUTHWESTERN ATLANTIC OCEAN

Bugoni L, Mancini PL, Monteiro DS, Nascimento L, Neves TS (2008a) Seabird bycatch in the Brazilian pelagic longline fishery and a review of capture rates in the southwestern Atlantic Ocean. ***Endangered Species Research.*** **In press.**

ABSTRACT

The southwestern Atlantic Ocean is an important foraging ground for several albatrosses and petrels during both the breeding and non-breeding seasons. In this region they meet longline fishing fleets, currently the main threat for this group of seabirds at sea. Here we present information on bycatch rates of seabirds in the Brazilian domestic pelagic longline fishery from 2001 to 2007 in the Exclusive Economic Zone (EEZ), and adjacent international waters of the southwestern Atlantic Ocean, and we review bycatch rates reported for the area. Overall seabird capture rate for the Brazilian pelagic longline fleet during 63 cruises (656 sets and 788,446 hooks) was 0.229 birds/1000 hooks, varying from zero to 0.542 birds/1000 hooks according to season. Capture rates were higher between June and November (cold season) and affected mainly the Black-browed Albatross *Thalassarche melanophris* (55% of birds captured), the White-chinned Petrel *Procellaria aequinoctialis*, the Spectacled Petrel *Procellaria conspicillata* and the Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*. Capture rates previously reported in the SW Atlantic varied from 0 to 5.03 birds/1000 hooks, with those based on logbooks or fishermen interviews tending to underestimate capture rates, whereas those based on small numbers of hooks or short time periods tend to greatly overestimate rates in both pelagic and demersal longline fisheries. In addition to more reliable data collected by onboard observers, those seabird-dedicated are able to collect a range of data about the circumstances of bycatch to further improve knowledge and mitigation. Previous studies had an important role in delimiting the seabird bycatch problem, and formed a baseline for mitigation actions. Furthermore, they serve as a guide for improvement in collecting data, more robust analysis and addressing further questions. The current study highlights the stochastic nature of seabird fatalities in longline fisheries and the need of extensive sampling for realistic estimation of capture rates, covering different years, seasons, vessels, and the range of fishing gear and practices. Longline fisheries are the major threat for albatrosses and petrels from different origins when foraging in the southwestern Atlantic Ocean, and require urgent conservation measures.

INTRODUCTION

The main reason for the current decline of many albatross and petrel populations around the world is bycatch in fisheries, particularly in pelagic longline fisheries (Gales 1997; Montevecchi 2002; BirdLife International 2004). Fishing activities could have short-term beneficial effects for seabirds, by bringing them otherwise unavailable food in the form of offal or discarded demersal fish and by removing competitors, or could have direct adverse effects when fisheries deplete stocks of prey of seabirds or drown them in nets or on longline hooks (Thompson & Riddy 1995; Montevecchi 2002; Furness 2003). In the medium to long-term or from a community perspective fishing discards appears to be detrimental for most species, for example by affecting other seabird species (Votier *et al.* 2004) or by providing low-quality fishery wastes which affect breeding performance

(Grémillet *et al.* 2008). For the Black-browed Albatross (*Thallassarche melanophris*) for example, trawling fishery is beneficial by providing discards close to breeding grounds, while pelagic longline in wintering grounds is detrimental due to incidental mortality (Rolland *et al.* 2008).

The impact of bycatch on seabird populations around the world is the focus of considerable international concern and incidental capture in longline fisheries is considered the most serious seabird-fishery issue for the conservation of several Procellariiformes (Gales 1997; Brothers *et al.* 1999; Furness 2003), being implicated in marked declines of several populations (Gales 1997; Tuck *et al.* 2001; Nel *et al.* 2002a; Cuthbert *et al.* 2004). Currently, 19 out of 21 albatross species are under risk of extinction (BirdLife International 2004). In the southwestern (SW) Atlantic Ocean different fisheries are detrimental to albatrosses and petrels, including trawling (Sullivan *et al.* 2006), gillnetting (Perez & Wahrlich 2005; Neves *et al.* 2006a), a range of artisanal or semi-industrial hook-and-line fisheries (Bugoni *et al.* 2008c), and industrial bottom and pelagic longlines (Neves & Olmos 1997; Favero *et al.* 2003).

The community of pelagic seabirds in the SW Atlantic Ocean and particularly off Brazil is dominated, in number of species and individuals, by albatrosses and petrels breeding in other areas such as Tristan da Cunha, Falkland (Malvinas) Islands, South Georgia, Antarctic and New Zealand, and feeding off Brazil during both the breeding and wintering periods (Olmos 1997; Neves *et al.* 2006b). This community shows seasonal variations, with low abundance in warm months and increasing abundance in cold months due to the arrival of Antarctic and sub-Antarctic migrants, which constitute the bulk of seabird on offshore waters (Olmos 1997; Neves *et al.* 2006a, b). The only Procellariiformes species breeding in Brazil are the Audubon's Shearwater (*Puffinus lherminieri*), with a few pairs on islands off the southeastern and northeastern coasts (Efe & Musso 2001), and the Trindade Petrel *Pterodroma arminjoniana* on Trindade Island (Luigi *et al.* 2008). In total, 10 albatross (Diomedeidae) and 31 petrel (Procellariidae, Hydrobatidae and Pelecanoididae) species are recorded in Brazil (CBRO 2007).

In this study we present data on capture rates for the Brazilian pelagic longline fishery from 2001 to 2007; abundance of seabirds attending longline vessels just before longline setting; we described the dynamic and magnitude of the pelagic fishing effort of the Brazilian domestic and leased fleets and its overlap with seabird distribution. In addition, a critical review of capture rates in longline fisheries reported in the SW Atlantic Ocean is provided. Gaps in our knowledge on seabird bycatch in the region, as well as conservation needs are discussed.

STUDY AREA AND METHODS

Data on seabird interactions with longline fisheries were collected in a large area over the Brazilian Exclusive Economic Zone (EEZ) and adjacent international waters, from 22ºS to 38ºS and 26ºW to 53ºW (Fig. 9.1). Fishing grounds in the SW Atlantic are under the influence of the Subtropical Convergence, where cold waters of the Falkland (Malvinas) Current flowing northward meet the warm waters of the Brazilian Current flowing southward (Garcia 1998). The presence of highly productive waters from the Falkland (Malvinas) Current in winter sustains an important pelagic longline fishery in southern Brazil (Castello 1998), as well as the most abundant and diverse seabird community in Brazil (Neves *et al.* 2006b). Over the Patagonian shelf, where the Falkland (Malvinas) Current flows, demersal longline fisheries occur. The separation between Brazilian and Falklands (Malvinas) Currents varies seasonally, from 35.8 to 38.6ºS, forming a heterogeneous area between both currents up to 1000 km wide with many eddies and fronts (Olson *et al.* 1988).

Brazilian pelagic longline fishery is composed basically by two distinct fleets (but see Neves *et al.* 2006a; Bugoni *et al.* 2008c) in terms of fishing gear characteristics and governmental management: the leased fleet composed of foreign medium to large vessels (30-50 m), which deploy longlines 40 to 55 nautical miles long, of monofilament nylon, with secondary lines about 15 m length without wire leaders, light-stick connected next to the hook 9/0 size or tuna hook, and using squid as bait. This fleet is mainly based at northeastern Brazilian ports, targeting Swordfish (*Xiphias gladius*), and tunas (*Thunnus* spp.) in a wide area of tropical and equatorial Atlantic Ocean, but some vessels could operate in the SW Atlantic Ocean during certain periods of the year. Vessels are able to operate continuously during the day and night due to large operational and storage capacity, staying at sea during several months and deployng more hooks than the national or domestic fleet (see Results for comparison between fleets). On their turn, the domestic fleet differs from the leased fleet by small size of the vessels (15-28 m), operational capacity limited to one month at sea, particularly due to captures stored in ice rather than frozen; deploy shorter mainline and usually 800 to 1200 hooks per set and a single deployment per day; the bait is usually squid, but also sardines and mackerel, with light-stick when the main target is Swordfish. This fleet also targets tunas and Swordfish, but Sharks (*Prionace glauca* and others) are a significant and sometimes the main part of captures, so a 0.5 m multifilament steel cable is attached at the terminal part of the secondary line near the hook. This fleet operates mainly at the shelf break of southern Brazil and adjacent international waters (see Results below for fishing grounds of both fleets).

Data for this study were collected by seabird observers during 63 pelagic longline fishing cruises from the Brazilian domestic fleet between January 2001 and November 2007,

departing from the main fishing ports of southern/southeastern Brazil of Santos, Itajaí and Rio Grande. Cruises lasted usually 15-25 days and observers collected data on seabird abundance, seabird bycatch and abiotic variables. Overall coverage was 656 sets and 788,446 hooks. The number of hooks deployed in each set varied from 230 to 1600 (mean = 1110 hooks).

Seabird abundance around fishing vessels was assessed through five counts just before dusk, with 15 minute intervals between counts, from 2001 to 2006. This method was adopted because most hooks are deployed at night (precluding census of seabirds), and was considered a suitable proxy of bird numbers attending the vessel before setting, the period of highest probability of bird capture (Brothers *et al.* 1999). Censuses were carried before the nautical sunset, i.e. the time when the centre of the sun is 12º below the horizon, to make comparable data collected in different areas and seasons. Seabird abundance in cold months (June-November, 221 censuses) and warm months (December-May, 62 censuses) is presented as frequency of occurrence (absolute and relative), and total and mean number of birds attending the vessel. Censuses were not carried at all settings due to safety onboard during bad weather or when the observer was less trained on seabird identification at sea. The maximum number of birds per species each day (i.e. each 5-counts sequence) was used for calculations. The division between cold and warm months is indicated by sea surface temperature off southern Brazil, which is thought to be a major determinant of seabird occurrence in the area (author's pers. observation).

Data on the domestic pelagic longline fishing fleet and the leased fleets from 2000 to 2006 were obtained from Brazilian reports of the regional fishery management organization ICCAT (International Commission for the Conservation of Atlantic Tuna), provided by the Standing Committee on Research and Statistics (available at http://www.iccat.int/Data/Assess/). The dynamic of both leased and domestic fleets in terms of variation in number of vessels from 2000 to 2006, and the spatial distribution of fishing effort as numbers of hooks deployed, exemplified by data of 2006, are presented (http://www.iccat.int/Data/Assess/MapHooksByFleet.rar).

A review of published and unpublished references reporting seabird capture rates in pelagic and demersal (=bottom) longline fisheries was carried out (Appendix 9.1). It covers studies in Brazil, Uruguay, Patagonian shelf and adjacent international waters, but was not exhaustive, particularly where there were several peer reviewed publications on the topic, such as the demersal fisheries on the Patagonian shelf area. Fishing effort was standardized as number of hooks deployed, and capture rates as birds/1000 hooks. As capture of seabirds in longline fisheries is a stochastic event we hypothesize that capture rates based on small number of hooks, and consequently limited number of trips or

during a single season, are not representative of the capture rates. Furthermore, it is reasonable to assume that studies with small or no captures are less prone to be published. Thus, a General Linear Model was carried out using the number of hooks and capture rates reported in a range of studies for both pelagic and bottom longline fisheries to test the hypotheses that estimates of capture rates based on limited sampling are usually overestimated. Capture rates and the number of hooks were transformed ($\ln x + 1$), looking for normality of residuals and homoscedasticity. Because demersal and longline fisheries have different orders of magnitude in reported capture rates and fishing effort, statistical analysis was separated between these fisheries. Publications that failed to report overall capture rates by focusing on a single species (e.g. Laich & Favero 2007) were excluded from statistical analysis. Statistical analysis was performed using Minitab® software, v. 15.

RESULTS

The Brazilian pelagic longline fishing fleet

Longline sets and seabird censuses sampled in this study were widespread in a large area over the Brazilian EEZ and adjacent international waters (Fig. 9.1), where most of the domestic Brazilian fleet target Swordfish, tunas and sharks (Fig. 9.2, Mayer & Andrade 2005). The overlap between observer coverage in this study (Fig. 9.1) and the fishing effort and fishing grounds of the pelagic longline fleets reported to ICCAT is particularly evident for the domestic fleet (Fig. 9.2). Overall, two important fishing grounds for the domestic fleet are located in the SW Atlantic, one along the southern Brazilian shelf break, and another in international waters over the Rio Grande Plateau (c. 33ºS, 32ºW, Figs. 9.1 & 9.2). Domestic and leased fleets also operate in northeastern areas, departing mainly from the port of Cabedelo, where overlap with the distribution of most seabird species is expected to be low. However, some of those vessels operate in southern fishing grounds during winter, a characteristic of this dynamic fleet that requires further investigation.

Comparing domestic and leased fleets, it is noticeable that the main effort of the leased fleet is in northeastern fishing grounds, and the domestic fleet concentrated in southern grounds (Fig. 9.2). Thus, the main overlap between seabird distribution and the pelagic longline occurs during winter from 20ºS southward, mainly, but not exclusively, with the national fleet, and is variable between years.

The variation in number of vessels of the pelagic longline fleet based in Brazil (leased and domestic) is presented in Figure 9.2. From 2000 to 2006, the number of vessels varied from 89 to 129, with a consistent decrease of the leased fleet and a slight increase of the domestic fleet (Fig. 9.3). However, the fishing effort in number of hooks deployed by Brazilian domestic and leased fishing fleets during this period ranged from 4.6 million

hooks in 2003 to 15.4 million in 2000. After 2003 there was an increase in fishing effort, and in 2006, the most recent year with information currently available, 9 million hooks were deployed, despite a decrease in number of vessels. The effort of the leased fleet was higher than that of the domestic fleet during all years, particularly in 2000 (92% of total), but also in 2006 when it represented 52%, despite accounting for only 1/3 of the vessels. This occurs because leased vessels are larger, deploy more hooks per set, operate continuously throughout the day, and have higher operational capacity, staying at sea for several months.

Seabird abundance

At least 21 seabird species were recorded attending Brazilian pelagic longline vessels (Table 9.1). Apart from occasional skuas and jaegers (genus *Stercorarius*) and frigatebirds, all other species were albatrosses and petrels, truly pelagic seabirds, as expected because sets occurred in offshore waters. Spectacled Petrels *Procellaria conspicillata* was the most abundant species attending longline fishing vessels off Brazil around the year, both by frequency of occurrence and number (Table 9.1). Atlantic Yellow-nosed Albatrosses *Thalassarche chlororhynchos*, Spectacled Petrels and Great Shearwaters *Puffinus gravis* showed similar abundance along the year, whereas seasonally abundant species during cold months were White-chinned Petrels *Procellaria aequinoctialis*, Cape Petrels *Daption capense*, Black-browed Albatrosses *Thalassarche melanophris* (mostly first-year juveniles), Southern Fulmars *Fulmarus glacialoides*, and Wilson's Storm-petrels *Oceanites oceanicus*.

Seabird capture in the Brazilian pelagic longline fishery

At least five seabird species were caught on fishing hooks during this study: Black-browed, Atlantic Yellow-nosed and Wandering *Diomedea exulans* Albatrosses, White-chinned and Spectacled Petrels (Table 9.2). A total of 178 birds were captured with a maximum of 40 birds in a single trip and 29 in a single set when 1225 hooks were deployed. Capture rates calculated by season and year varied from 0 to 0.542 birds/1000 hooks with higher capture rates during the cold season (Table 9.3). The capture rates were variable between trips, with half of the trips resulting in no captures (31 out of 63 trips), and only 61 out of 656 sets (11%) capturing birds. Overall, a capture rate of 0.229 birds/1000 hooks was recorded, and the Black-browed Albatross was the most common species caught, with 55% of captured birds, followed by the White-chinned Petrel with 26% (Table 9.2). Capture rate of the Spectacled Petrel was ranked only fourth in spite of being the most frequent and abundant species attending vessels. Birds are captured predominantly during cold months, when there is greater overlap in southern Brazil between large numbers of species more prone to be captured, such as the Black-browed Albatross and the White-chinned Petrels, with higher fishing effort of the pelagic longline fleet.

Seabird capture rates reported for the SW Atlantic

Seabird capture rates in the SW Atlantic have been reported in the literature from 1991 to 2007. At least 28 studies focused on incidental capture of albatrosses and petrels in demersal (17 studies) and pelagic (16 studies) longlines (Appendix 9.1). Sampling methods included analysis of logbooks, interview with fishing skippers or other fishermen, observers primarily dedicated to collect fishery data rather than seabird data making occasional observations of seabird bycatch, and seabird-dedicated onboard observers. The reliability of data obtained by each method is obviously variable, but biases associated with methods are difficult to evaluate, due to the difficulty of disentangling different factors and because some studies failed to provided sufficient accurate details. For the Brazilian pelagic longline fishery there is a trend for lower capture rates in the dataset provided by fishermen through logbooks or interview. Data obtained by logbooks or interview tended to have higher coverage (i.e. more hooks sampled), but their reliability is difficult to assess. Fishing effort reported was also very variable from a few thousands up to 150 million hooks. Demersal longline studies in general reported lower mean capture rates (0.005 to 0.41 birds/1000 hooks) and were more consistent in having less variation in capture rates and by relying on samples with higher fishing effort. On the other hand, in the pelagic longline fishery the capture rates varied among studies from zero to 5.03 birds/1000 hooks, with variability within some studies, including some highly skewed values (Fig. 9.4).

Negative correlations between observed fishing effort (measured as number of hooks set) and capture rates were found for the demersal ($F_{1, 9} = 10.08$, $P = 0.01$) and pelagic ($F_{1,10} = 14.56$, $P = 0.003$) fisheries in the SW Atlantic Ocean (Fig. 9.4). Variation in number of hooks explained 45.2% of the variation in capture rates in the demersal fishery and 53.0% in the pelagic fishery. This result suggests that studies based on low numbers of hooks often greatly overestimate seabird capture rates.

DISCUSSION

Seabird capture in the Brazilian pelagic longline fishery

Several species of albatrosses and petrels are captured by the Brazilian pelagic longline fishery and apart from those reported here (Table 9.1), Tristan *Diomedea dabbenena*, Southern royal *D. epomophora*, and Northern royal *D. sanfordi* Albatrosses, Great Shearwaters and Southern Fulmars are known to be affected (Vaske-Jr 1991; Neves & Olmos 1997; Olmos *et al.* 2001). In the present study Black-browed Albatrosses and White-chinned Petrels showed the highest capture rates, in agreement with previous studies across the whole SW Atlantic waters, in both pelagic and demersal longline fisheries. In contrast, Atlantic Yellow-nosed Albatross and Spectacled Petrel are captured particularly in Uruguay and Brazil (this study; Jiménez & Domingo 2007), coinciding with a more northern distribution of these species in the SW Atlantic. On the other hand, the

high abundance of Spectacled Petrels attending fishing vessels contrast with its limited capture rate, as it was ranked fourth in number of fatalities

Capture rates reported here varied greatly according to seasons, trips, and sets, an inherent characteristic of captures, where only a few sets capture birds and most have no capture (91% in this study, 85% in Brothers *et al.* 1999; Reid & Sullivan 2004).

Seabird bycatch in the SW Atlantic Ocean

Incidental mortality of seabirds in the SW Atlantic waters was early reported by Croxall & Prince (1990) through band recoveries of Wandering Albatrosses breeding in South Georgia Islands and killed by the tuna longline fishery off South America. However, capture rates were first calculated in 1987-90 (Vaske-Jr 1991). This and other early studies of capture rates reported high values, such as 1.35 birds/1000 hooks (Vaske-Jr 1991; Appendix 9.1) in the tuna longline fishery in southern Brazil during winter and spring (=cold months in this study) or 4.7 birds/1000 hooks for the Uruguayan fleet operating in the same area and with no weighted swivels (Stagi *et al.* 1997). However, high capture rates are probably due to lower number of hooks analysed, particular features of the fishing gear and cruises taking place in winter, when large numbers of species vulnerable to capture are present in the area. During the last two decades, at least 28 studies focused on the incidental capture of seabirds in demersal and pelagic longline fisheries in Brazil, Uruguay, Patagonian shelf and Falkland (Malvinas) Islands (Appendix 9.1). These studies encompass an area from southeastern Brazil (c. 20°S) to sub-Antarctic waters (c. 58°S) and include a wide range of fishing methods, targeted species, sampling methods, sampling effort and accuracy of data. Most studies with large numbers of hooks sampled were obtained from logbooks, fishermen interviews or non-dedicated seabird observers. For both demersal and pelagic fisheries, captures based in limited sampling (i.e. small number of hooks) tend to report higher capture rates (Fig. 9.4). Early studies were fundamental in identifying the problem, species, area and fisheries where captures occurred and also illustrate the range of situations that determine high captures. Currently, estimations of numbers of birds killed will greatly be benefited from robust data obtained by dedicated seabird observers, larger and representative datasets, and analysed by using more robust statistics, such as multi-stage analysis with stratified data (e.g. in Reid *et al.* 2004; Laich & Favero 2007 for the demersal longline fishery), rather than simplistic extrapolations of capture rates and total fishing effort for a given fleet or area. It is of interest of all and responsibility of regulatory organizations and governments to provided adequate conditions for obtaining more rigorous data. The importance of large data sets and reliable data collected by onboard observers is evident in the work by Jiménez (2005) and Jiménez & Domingo (2007) where circumstances of incidental captures were identified: the shelf break off Uruguay as an area of high incidental mortality, as well as hook setting during daylight

hours or bright moon phases, and cold waters, as important factors accounting for seabird mortality. It is surprising that even basic data such as numbers of hooks, season, or type of longline, are frequently not reported in capture rate estimates (Appendix 9.1). However, there is a general improvement in recent datasets and the use of analytical tools, since the pioneering study of Vaske-Jr (1991). Thus, considering the several capture rates for the pelagic longline fisheries (Appendix 9.1), those based on large datasets, several cruises, vessels, fishing gear, areas, and collected by seabird-dedicated onboard observers appear to place representative annual estimates of captures in the range of 0.2 to 0.4 birds/1000 hooks.

Estimates of population-level impacts are expected to be greatly influenced by the values used in the model (Inchausti & Weimerskirch 2001; Hyrenbach & Dotson 2003; Lewison *et al.* 2005). Lewison *et al.* (2005) and Lewison & Crowder (2007) discuss the problems associated with incidental capture rates and their limited reliability for estimation of number of individuals captured for a fleet or an ocean basin. Additionally, this sort of exercise suffers from the high dynamism of longline fleets, which vary spatially, temporally and in size (Hyrenbach & Dotson 2003; Tuck *et al.* 2003; Mayer & Andrade 2005; this study). For instance, the number of vessels of the pelagic longline fleets based in Brazil (leased and domestic) is also variable between years (Fig. 9.3) and highly dynamic, which have important implications for the capture of albatrosses and petrels and their mitigation.

Overlap between fishery and seabirds and implications for conservation

Black-browed and Atlantic Yellow-nosed Albatrosses, Spectacled and White-chinned Petrels, and Great Shearwaters were the most numerous seabirds attending pelagic longliners in Brazil. This pattern was expected and corroborates previous information on seabirds attending longline vessels in Brazil (Olmos 1997; Olmos & Bugoni 2006; Neves *et al.* 2006b). The Spectacled Petrel is endemic to Inaccessible Island, Tristan da Cunha group, and has a small breeding population estimated at 20,000 adult birds (Ryan *et al.* 2006). The bulk of the Spectacled Petrel population appears to concentrate off Brazil and Uruguay, as it is rare off South African wintering grounds (Camphuysen & van der Meer 2000; Camphuysen 2001). In the SW Atlantic, Spectacled Petrels are probably immature birds as well as breeding birds performing long foraging trips, as recorded for the foraging trips of White-chinned Petrels (Weimerskirch *et al.* 1999). The Atlantic Yellow-nosed Albatross had high frequency of occurrence in cold months (63%), but with small numbers attending vessels. This species has also a small breeding population endemic to the Tristan da Cunha Archipelago (Cuthbert & Sommer 2004) and immature as well as mature birds, differentiated by bill colours and plumage moult, are found in the SW Atlantic throughout the year and are captured in a range of fisheries (this study; Bugoni *et al.* 2008a). The only other species abundant throughout the year is the Great

Shearwater, though found in largest flocks during autumn, during their journey to the North Atlantic Ocean where they winter, and again in spring, during their southward migration to Tristan da Cunha breeding grounds (Metcalf 1966; Neves *et al.* 2006b), or birds using the area for moult (Chapter 5). During cold months, southern migrants reach southern Brazilian waters, particularly the Black-browed Albatross and the White-chinned Petrel (Phillips *et al.* 2006), which are the most abundant species in the area as well as the most captured within this period. In other areas the capture in summer (=breeding period) is higher than in cold months (Brothers *et al.* 1999), confirming that most albatrosses and petrels have neither safe area nor season, being killed by pelagic and bottom longline throughout the year and in many different areas. The SW Atlantic Ocean is an important area for juvenile Black-browed Albatrosses from Falkland (Malvinas) Islands, but also for adults of this and other populations (BirdLife International 2004; Phillips *et al.* 2005b). Severe decline of the Black-browed Albatross global population is attributed to pelagic longline fisheries in the SW Atlantic, as well as a range of other fisheries (Sullivan *et al.* 2006; Neves *et al.* 2006a, 2007; Bugoni *et al.* 2008c).

The displacement of the leased fleet to northeast Brazil during summer months (Fig. 9.4) shows minor overlap with albatross and petrel distribution during this period, which could suggest negligible capture rates. However, the operation of some leased vessels in southern waters is of concern. Furthermore, preliminary data collected by onboard observers (non-seabird dedicated) in 2005 shows high numbers of Great Shearwaters, as well as lower numbers of threatened species, being captured and raises concern due to the high fishing effort in this area and available reports of a range of species being captured (Neves *et al.* 2007). There is no study on capture rates on the fleet based in northeastern Brazilian ports, which requires urgent study. The dynamic of pelagic longline fleets in Brazil and elsewhere (Hyrenbach & Dotson 2003) suggests that continuous monitoring of the fleets is necessary. Non-negligible capture rates of Spectacled Petrels, Atlantic Yellow-nosed, Tristan and Wandering Albatrosses in southern Brazil and Uruguay (Jiménez & Domingo 2007; this study) are a major concern due to their small global populations. Also abundant in summer, these species are affected by the longline fishing during this time of the year, when the Brazilian fishing fleet is active over a larger area along the southeastern Brazilian waters, but also over the Rio Grande Plateau in summer.

Improving data on capture rates in general and in poorly studied fisheries is recommended, particularly those using hook-and-line methods in Brazil (Bugoni *et al.* 2008c) and the northeastern leased fleet. For better studied fleets, such as the national pelagic fleet based on southern Brazilian ports, detailed analysis of environmental factors and fishing conditions affecting the incidental capture of seabirds is compelling in order to delineate effective conservation strategies. In addition, the implementation of actions

suggested in national plans of action (NPOA-Seabirds) available for all jurisdictional countries in the SW Atlantic is urgently required given the well established nature of the problem and their significance in the decline of several species. Despite recent studies based on large datasets reporting lower capture rates for the pelagic longline in comparison with early reports, this is probably due to better sampling coverage and not to improvement in mitigation of captures and conservation actions (but see e.g. Otley *et al.* 2007b for the bottom longline). Capture rates available in a range of fisheries and throughout the SW Atlantic are well above sustainable or acceptable levels.

Table 9.1. Abundance of seabirds attending longline fishing vessels off Brazil measured by frequency of occurrence (FO) and relative frequency (FO%), mean and total number of birds between 2001 and 2006. Values were obtained from the maximum number counted in each census-day just before dusk. Cold months are from June to November (n = 221 censuses) and warm months from December to May (n = 62 censuses).

	June-November				December-May			
	FO	FO%	Nr	Mean No.	FO	FO%	Nr	Mean No.
Spectacled Petrel *Procellaria conspicillata*	191	86.4	1929	8.7	41	66.1	277	4.5
White-chinned Petrel *Procellaria aequinoctialis*	103	46.6	638	2.9	15	24.2	31	0.5
Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*	139	62.9	456	2.1	21	33.9	41	0.7
Great Shearwater *Puffinus gravis*	101	45.7	432	2.0	35	56.5	163	2.6
Cape Petrel *Daption capense*	88	39.8	427	1.9	3	4.8	4	<0.1
Black-browed Albatross *Thalassarche melanophris*	97	43.9	372	1.7	19	30.6	38	0.6
Southern Fulmar *Fulmarus glacialoides*	14	6.3	86	0.4	0	0	0	0
Manx Shearwater *Puffinus puffinus*	5	2.3	99	0.4	2	3.2	3	<0.1
Wilson's Storm-petrel *Oceanites oceanicus*	38	17.2	72	0.3	1	1.6	1	<0.1
Large albatrosses *Diomedea* spp. (including *dabbenena/exulans*)	24	10.9	49	0.2	0	0	0	0
Atlantic Petrel *Pterodroma incerta*	14	6.3	28	0.1	4	6.5	5	<0.1
Southern Royal Albatross *Diomedea epomophora*	2	0.9	2	<0.1	0	0	0	0
Northern Royal Albatross *Diomedea sanfordi*	2	0.9	2	<0.1	0	0	0	0
Soft-plumaged Petrel *Pterodroma mollis*	1	0.5	1	<0.1	0	0	0	0
Unidentified *Pterodroma* sp.	1	0.5	3	<0.1	0	0	0	0
Sooty Shearwater *Puffinus griseus*	1	0.5	1	<0.1	0	0	0	0
Cory's Shearwater *Calonectris diomedea*	5	2.3	14	<0.1	0	0	0	0
Southern Giant Petrel *Macronectes giganteus*	6	2.7	11	<0.1	0	0	0	0

Table 9.1. Continued

Northern Giant Petrel *Macronectes halli*	1	0.5	1	<0.1	0	0	0	0
Giant Petrels *Macronectes* sp.	4	1.8	5	<0.1	0	0	0	0
White-bellied Storm-petrel *Fregetta grallaria*	1	0.5	1	<0.1	0	0	0	0
Skuas and Jaegers *Stercorarius* spp.	2	0.9	3	<0.1	0	0	0	0
Frigatebirds *Fregata* sp.	2	0.9	2	<0.1	0	0	0	0

Table 9.2. Capture rate of seabird species in the domestic pelagic longline fishery in Brazil from 2001 to 2007 (n = 788,446 hooks).

Species	Capture rate (birds/1000 hooks)
Black-browed Albatross *Thalassarche melanophris*	0.126
Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*	0.011
Unidentified *Thalassarche* albatrosses	0.018
Wandering Albatross *Diomedea exulans*	0.001
Unidentified *Diomedea* albatrosses	0.005
White-chinned Petrel *Procellaria aequinoctialis*	0.059
Spectacled Petrel *Procellaria conspicillata*	0.008
Overall capture rate	0.229

Table 9.3. Seasonal and inter-annual variation in capture rates of seabirds (birds/1000 hooks) in the domestic pelagic longline fishery of Brazil, from 2001 to 2007. Standard deviation (SD) of capture rates were calculated from a binomial probability distribution, taking into account the number of hooks deployed and number of birds captured. Overall capture rate = 0.229 birds/1000 hooks, and number of hooks deployed = 788,446 hooks. --- Not sampled.

	Warm months (December-May)		Cold Months (June-November)	
	Capture Rate ± 1 SD	No. hooks	Capture Rate ± 1 SD	No. hooks
2001	0.000	23,893	0.000	36,900
2002	0.217 ± 0.063	55,400	0.177 ± 0.059	50,900
2003	---	---	0.124 ± 0.051	48,400
2004	0.000	5,400	0.036 ± 0.021	82,958
2005	0.000	39,190	0.129 ± 0.032	123,940
2006	0.073 ± 0.051	27,390	0.415 ± 0.088	53,045
2007	0.142 ± 0.050	56,460	0.542 ± 0.054	184,570

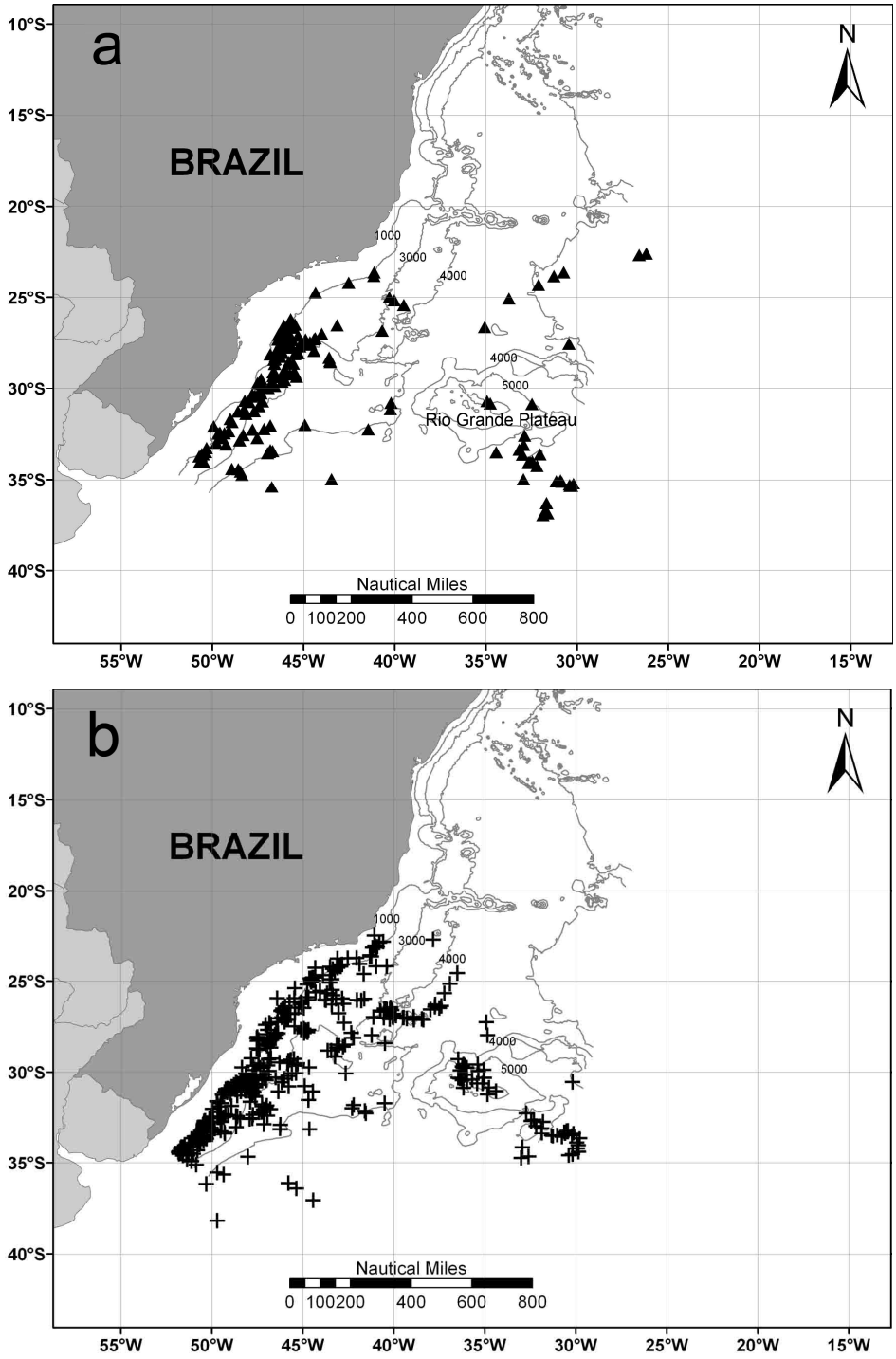

Figure 9.1. Locations of 656 pelagic longline settings for the domestic fleet off Brazilian coast sampled by onboard seabird observers from 2001 to 2007. Warm months are from December to May, n = 176 sets (a); cold months are from June to November, n = 480 sets (b). The separation between the Brazilian Current flowing southward and the Falkland (Malvinas) Current flowing northward varies annually and seasonally, from 35ºS to 39ºS.

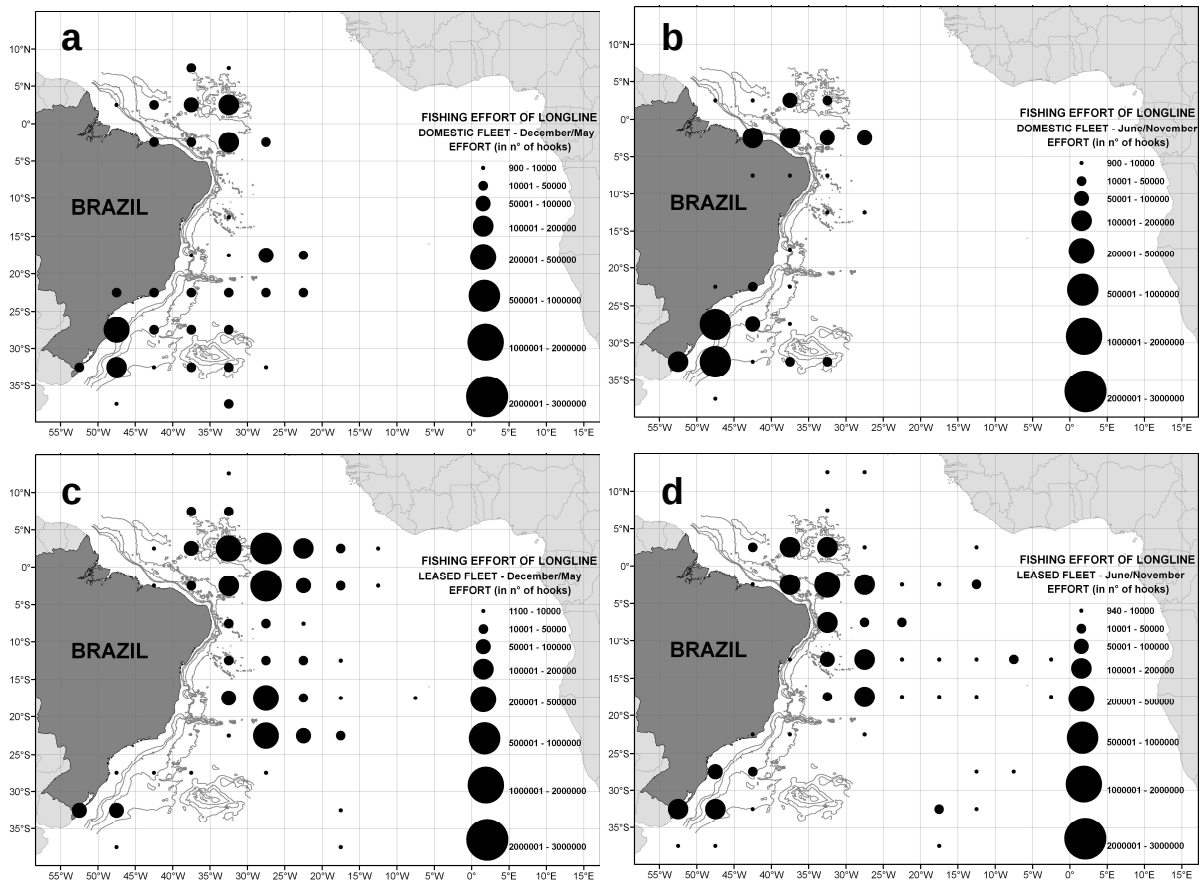

Figure 9.2. Spatial distribution of effort of the Brazilian domestic pelagic longline fishing fleet in 2006 (latitude-longitude resolution of 5° x 5°) during warm months from December to May, 1.5 million hooks (a); and cold months from June to November, 3.2 million hooks (b); and the leased fleet during warm months from December to May, 3.2 million hooks (c); and cold months from June to November, 1.9 million hooks (d).

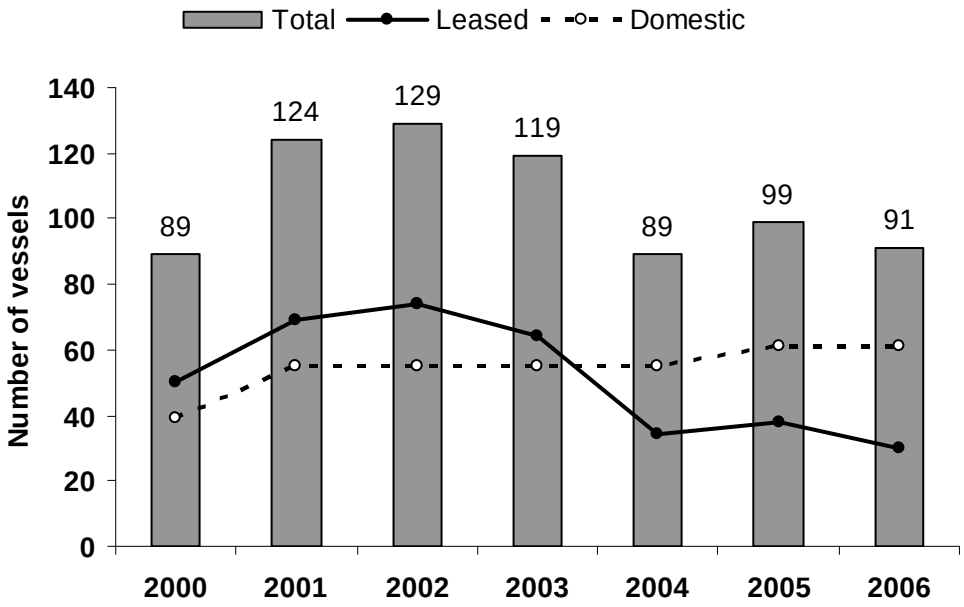

Figure 9.3. Annual variation in the number of pelagic longline vessels based in Brazilian ports from 2000 to 2006 for the domestic and leased fleets. Total number of vessels is shown above the bars.

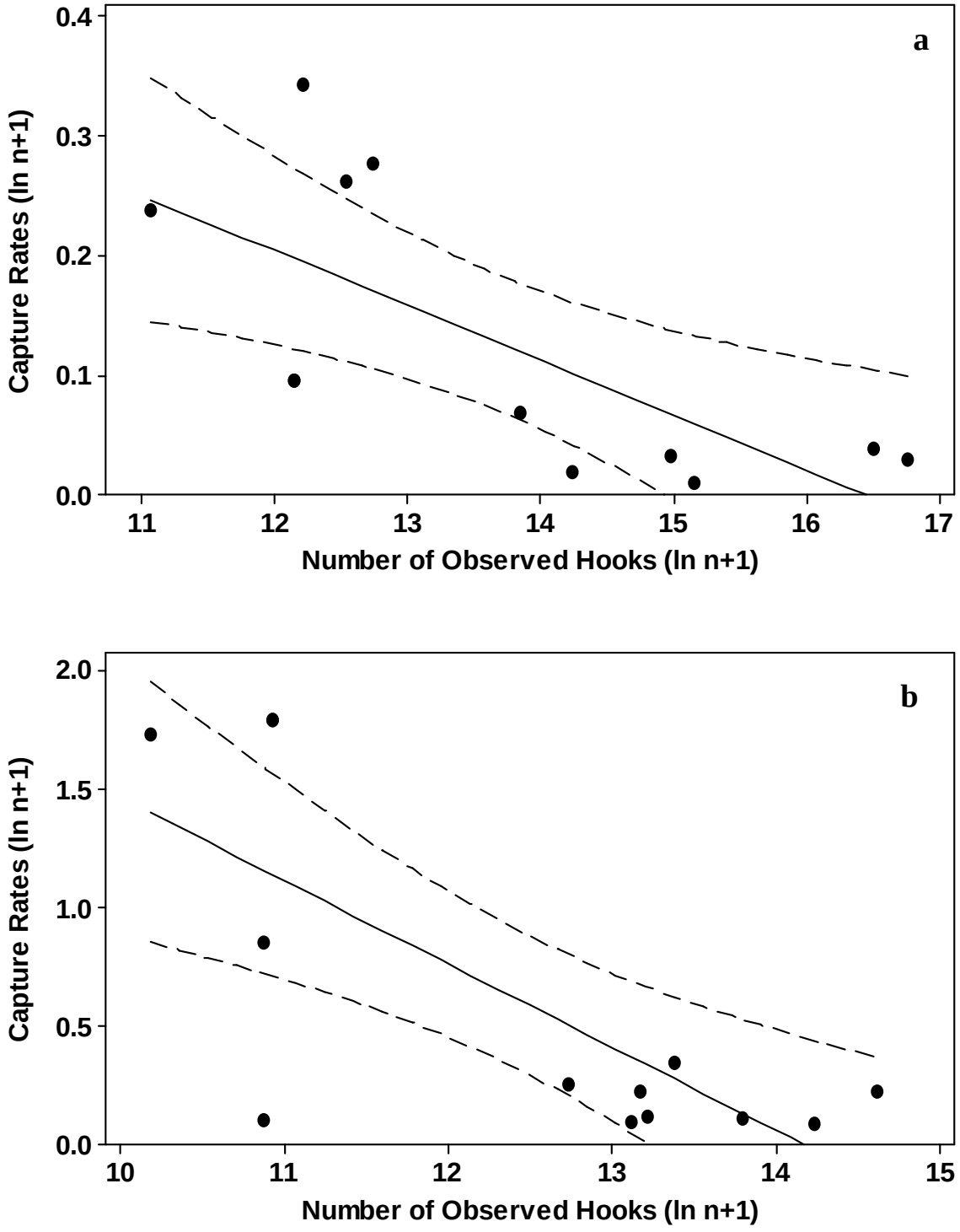

Figure 9.4. Correlation between number of hooks and capture rates measured by birds/1000 hooks for the demersal (a) and pelagic (b) longline fisheries. Values are transformed (Log n+1), trend line and 95% confidence intervals are shown.

Appendix 9.1. Summary of studies reporting capture rates of seabirds (birds/1000 hooks) in demersal and pelagic longline fisheries in the southwestern Atlantic Ocean from 1991 to 2008. (---) Data not provided.

Longline Type	Location	Mean Capture Rate	Range Capture Rate*	Year(s)	No. of Hooks Observed	Sampling Method	Comments	References
Demersal for Patagonian Toothfish and Hake	Argentina	---	---	1993-1995	25,386,000	Log books and interview	Anecdotal data on bycatch.	Schiavini *et al.* (1997)
Demersal for Toothfish and Kingclip	Argentina - Patagonian shelf	0.04	0-0.2	1999-2000	~14.8 millions	Non dedicated onboard observers	Steep decline in capture rate during years of the study; 99% of sets with mitigation measures.	Favero *et al.* (2003)
Demersal for Kingclip	Argentina - Patagonian shelf	0.034	SD=0.009	2000-2001	3,193,944	Seabird dedicated observers	Moon phase and water depth explained most of captures.	Gandini & Frere 2006)
Demersal for Kingclip, Patagonian toothfish and Yellownosed Skate	Argentina - Patagonian shelf and shelf break	0.03	0.001-0.18 (SD=0.39)	1999-2003	19,067,100	Non dedicated onboard observers		Laich *et al.* (2006)
Demersal for Kingclip, Patagonian	Argentina - Patagonian shelf and	0.014[1]	(SD=0.090)	1999-2003	Not explicit, but stated to be 30	Non dedicated onboard	[1]Only analyzed for White-chinned Petrel. Environmental variables	Laich & Favero (2007)

toothfish and Yellownosed Skate	shelf break				millions per year, thus ~150 millions	observers	affecting captures were detected.	
Demersal for Kingclip	Argentina - Patagonian shelf	0.071	0.034-1.53	2005	1,033,900	Onboard observers?	Single vessel, summer.	Seco-Pon *et al.* (2007)
Demersal for Patagonian Toothfish	Falkland (Malvinas) Is. - Patagonian shelf	0.019	0-0.032	2001-2002	1,523,155	Dedicated and non-dedicated onboard observers	2 to 4 tori-lines used.	Reid *et al.* (2004) Reid & Sullivan (2004)
Demersal for Patagonian Toothfish	Malvinas/ Falkland (Malvinas) Is. - Patagonian shelf	0.010	0.-8.504	2202-2204	~17.1 millions	Dedicated onboard observers	2 to 3 tori-lines used; injury and delayed mortality by lost hooks reported.	Otley *et al.* (2007b)
Pelagic for tunas	Uruguay – off Brazil & Uruguay	5.03	---	1994	55,624	---		Barea *et al.* (1994)
Pelagic for tunas	Uruguay	4.7	0-481.3	1994	26,364	Onboard observers	Capture rate of 481.3 birds/1000 hooks was based in a set of only 320 hooks.	Stagi *et al.* (1997)
Demersal for rays and other spp.	Uruguay	0.41	0.075-0.575	1995	202,650	Onboard observers	Only two cruises sampled.	Stagi *et al.* (1997)

Not provided - Probably pelagic	Uruguay	1.7	---	---	1.5 million	---	Anecdotal data (no methods, fleet or birds caught reported).	Stagi & Vaz-Ferreira (2000)
Pelagic for tuna, Swordfish and sharks	Uruguay and International waters	---	0.05-5.57[2]	1993-1996	155,040	Onboard observers	[2] Capture rate calculated for non-fish (birds, mammals and sea turtles).	Marín *et al.* (1998)
Semi-pelagic (=demersal) for Wreckfish	Uruguayan EEZ	3.0	---	2001	---	Onboard observers		Marín *et al.* (2004)
Pelagic for Swordfish, tunas and sharks	Uruguay and International waters	0.42	0.04-1.65	1998-2004	647,722	Dedicated and non-dedicated onboard observers		Jiménez (2005) & Jiménez *et al.* (2005)
Pelagic for Swordfish, tunas and sharks	Uruguay and International waters	0.26		1998-2006	2,242,026	Dedicated and non-dedicated onboard observers	Monthly capture rates provided. Higher in southern area and winter.	Jiménez & Domingo (2007)
Pelagic for tunas	Southern Brazil	1.35	0-97.9	1987-1990	52,593	Onboard observers	Winter months; high capture rates during stormy weather; higher capture rate (97.9) calculated from a set of only 1,205 hooks;	Vaske-Jr (1991), and pers. comm. on total number of hooks.

							several authors erroneously derived capture rates from Vaske's paper based only on sets with bird captures.	
Pelagic for tunas, Swordfish and sharks	Brazil and adjacent international waters	0.12	---	1994-1995	*c.* 983,333	Log books	Capture rate considered underestimate and highly variable.	Neves & Olmos (1997)
Demersal for Tilefish, Namorado and groupers	Brazil	0.3	---	1994-1995	280,197	Log books	Research vessel; Capture rate included 49 unidentified birds.	Neves & Olmos (1997)
Demersal	Brazil	---	0.1-0.32	---	---	Onboard observers	Review of two other studies.	Olmos *et al.* (2000)
Pelagic for Swordfish	Brazil	---	0.09-1.35	---	---	Onboard observers	Data are from three previous studies.	Olmos *et al.* (2000)
Demersal for Tilefish, Namorado and groupers	Brazil	0.32	---	1994-1995	340,777	Log books and onboard observers	Research vessel; data partially reported in Neves & Olmos (1997).	Olmos *et al.* (2001)

Demersal for Tilefish, Namorado and groupers	Brazil	0.1	---	1996-1997	187,908	Log books	Research vessel.	Olmos *et al.* (2001)
Pelagic for tunas, sharks and Swordfish	Brazil	---	0.095-0.73	1994-1999	1,529,312	Interview and onboard observers	Data partially reported in Neves & Olmos (2001); include data from research vessel.	Olmos *et al.* (2001)
Demersal for Tilefish, Namorado, groupers, etc.	Brazil	0.26	0.1-0.32	1994-1997	528,685	Fishermen interview		Neves *et al.* (2001)
Pelagic for tunas, Swordfish and sharks	Brazil	0.095	---	1994-1999	1,529,312	Fishermen interview		Neves *et al.* (2001)
Demersal for Tilefish, Namorado, groupers, etc.	Brazil	0.298	---	1994-1995	338,812	Onboard observers	Research cruises.	Tutui *et al.* (2000)
Pelagic for tunas, Swordfish and sharks	Brazil	0.27	0-6	2002-2003	64,150	---	Only five cruises; use of mitigation measures; cite other three previous cruises with higher capture rates	Soto *et al.* (2003)

							without details, and no cruise with 'zero' capture rate reported.	
Demersal	Brazil	0.101	---	1996-1997	188,000	Onboard observers	Research cruises.	Vooren & Coelho (2004)
Pelagic for Swordfish, tunas, sharks	Brazil	0.102	---	2000-2005	499,978	Onboard observers	Capture rates for demersal longline based in previous studies	Neves *et al.* (2007)
Pelagic for Swordfish and Dolphinfish	Brazil	0.114	0-0.15	2001-2006	52,691	Onboard observers	Small vessels from Itaipava fleet; focused on the description of other fisheries.	Bugoni *et al.* (2008c)
Pelagic for tunas, Swordfish and sharks	Brazil and international waters	0.229	0-0.542	2001-2007	778,446	Onboard observers	No mitigation measure.	This study

* Range of capture rates was reported in several ways, e.g. between sets, cruises, season or areas.

Common names: Namorado (*Pseudopercis numida*), Tilefish (*Lopholatilus villarii*), Groupers (*Epinephelus* spp.), Toothfish (*Dissostichus eleginoides*), Hake (*Merluccius hubbsii*), Kingclip (*Genypterus blacodes*), Tunas (*Thunnus* spp.), Swordfish (*Xiphias gladius*), Sharks (several species, including *Prionace glauca*, *Sphyrna* spp., *Carcharhinus* spp., and *Alopias* spp.), Wreckfish (*Polyprion americanus*), Yellownosed skate (*Dipturus chilensis*), Dolphinfish (*Coryphaena hippurus*).

Chapter 10

POTENTIAL BYCATCH OF SEABIRDS AND TURTLES IN HOOK-AND-LINE FISHERIES OF THE ITAIPAVA FLEET, BRAZIL

Bugoni L, Neves TS, Leite-Jr NO, Carvalho D, Sales G, Furness RW, Stein CE, Peppes FV, Giffoni BB, Monteiro DS (2008c) Potential bycatch of seabirds and turtles in hook-and-line fisheries of the Itaipava Fleet, Brazil. ***Fisheries Research*** 90(1–3): 217–224.

ABSTRACT

The decline of populations of certain seabirds and sea turtles around the world is partly related to their incidental capture in large-scale fisheries. However, the impacts of small-scale fisheries on endangered seabirds and sea turtles, being carried out in many places around the world, have been largely neglected by scientists and governments. We monitored 178 fishing days and described a range of poorly known hook-and-line commercial fisheries carried out by the Itaipava fleet, southeastern Brazil, composed by 497 vessels and deploying hooks from 18°S to 35°S. Seven fisheries were defined: fast trolling for tuna and tuna-like species, slow trolling for Bigeye Tuna, handlining, surface longline for Dolphinfish, pelagic longline for Swordfish, bottom dropline, and pole-and-line with live bait. We observed bycatch of 47 seabirds of six species and 45 turtles of four species. Capture rates were higher for the surface longline for Dolphinfish (0.15 birds/1000 hooks and 1.08 turtles/1000 hooks), slow trolling for Bigeye Tuna (0.41 birds/day) and handlining targeting Yellowfin Tuna (0.61 birds/day). Endangered Spectacled Petrel (*Procellaria conspicillata*), Atlantic Yellow-nosed (*Thalassarche chlororhynchos*), and Black-browed (*T. melanophris*) Albatrosses were the main seabirds caught. Immature Loggerhead Turtles (*Caretta caretta*) and immature or adult Leatherback Turtles (*Dermochelys coriacea*) were the main sea turtles affected by the surface longline for Dolphinfish. Monitoring the fleet and bycatch levels, development of mitigation measures, establishment of educational programs, government control over the fleet, and enforcement, are urgently required for the hook-and-line fisheries described in the present study.

INTRODUCTION

Fisheries are a major cause of mortality for seabirds and sea turtles around the world (National Research Council 1990; Brothers *et al.* 1999), accounting for the decline of several species (Gales 1997; Lewison *et al.* 2004). An important cause of seabird mortality is the interaction with pelagic longlines for Tunas (*Thunnus* spp.) and Swordfish (*Xiphias gladius*), which have received much attention of scientists (e.g. Brothers *et al.* 1999; Gales 1997). Fisheries such as gillnet, trawling, and dropline have been considered a minor mortality factor for pelagic seabirds (Gales 1997), but recent studies show that some other fisheries cause high mortality levels. Around Falkland (Malvinas) Islands, Sullivan *et al.* (2006) estimated a mortality rate of 0.47 seabirds per fishing day per vessel in the factory trawl fleet for finfish. Gillnets targeting Monkfish (*Lophius gastrophysus*) off the Brazilian coast were estimated to kill 802 petrels and albatrosses in 2001 (Perez & Wahrlich 2005).

Trawl, gillnet and longline are the main fisheries reported to capture sea turtles (National Research Council 1990; Oravetz 1999). Trawling for fish and shrimp is estimated to kill 150,000 turtles annually around the world, mostly Loggerhead (*Caretta caretta*),

Leatherback (*Dermochelys coriacea*) and Green (*Chelonia mydas*) Turtles (Oravetz 1999), while pelagic longlines captured in 2000 an estimated 200,000 Loggerheads and 50,000 Leatherbacks (Lewison *et al.* 2004). Despite several uncertainties regarding these estimates, they give an approximation of the global impacts on sea turtles.

In the Southwestern (SW) Atlantic Ocean, which encompasses waters off Brazil, Uruguay, Argentina, and adjacent international waters, detailed studies on seabird bycatch have focused on pelagic and demersal longlines (Neves & Olmos, 1997; Seco-Pon *et al.* 2007), while few studies considered other fisheries (e.g. Perez & Wahrlich 2005). Neves & Olmos (1997) reported 0.12 birds/1000 hooks in the pelagic longline fishery mostly Black-browed Albatross, Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*) and White-chinned Petrel (*Procellaria aequinoctialis*). Although seabird mortality is historically related to the longline fishery (pelagic and bottom) carried out by the domestic and leased fleet, other fisheries - such as pole-and-line with baitboats, gillnetting, trawling and drift netting have potentially relevant incidental capture rates and must be evaluated (Neves *et al.* 2006a). Regarding sea turtles, a range of fisheries are also important mortality factors (Bugoni *et al.* 2001) and at least 13 fisheries were identified to capture sea turtles in the SW Atlantic Ocean (Domingo *et al.* 2006), but detailed information exists only for a few fisheries, particularly pelagic longline, in which bycatch varies from 0.68 to 2.85 turtles/1000 hooks (Domingo *et al.* 2006).

The SW Atlantic waters have an important role in the life cycle of five sea turtle species nesting in Brazil, as well as migrating sea turtles from other areas, such as Leatherback Turtles from Gabon, the second largest nesting ground in the world, and Green Turtles from Ascension Island, both migrating to the area after nesting (Domingo *et al.* 2006). Similarly, Brazil holds important populations of albatrosses and petrels which breed in Antarctic and Sub Antarctic Islands, Patagonia, Tristan da Cunha and Gough Islands, New Zealand, British Isles, Azores, Madeira and Cape Verde Islands (Neves *et al.* 2006b). Some species are found in the area during non-breeding periods, while others perform long foraging trips during breeding to fish in Brazil and feed chicks in remote islands.

While most studies in Brazil and elsewhere have focused on large-scale fisheries, small-scale or artisanal fisheries could also have impacts on seabirds and sea turtles, but have been neglected by scientists and regulatory agencies. For instance, there is a large high sea pelagic fleet in Itaipava port, a small village on the Espírito Santo coast, southeastern Brazil which originated in 1988, after the collapse of coastal resources targeted using artisanal methods and small vessels. Currently, the fleet is composed of 497 vessels up to 14 m long, targeting tunas, Dolphinfish (*Coryphaena hippurus*), and Swordfish, as well as bottom rocky and reef fishes, and using a range of artisanal hook-and-line gears and techniques (Martins *et al.* 2005). Fishing methods used by the

Itaipava fleet have not been described and there is no regulation or management by the government. The size of the fleet and methods used, associated with fishermen's reports of seabirds and sea turtles frequently being captured make it a major conservation concern.

The present paper aims to describe several poorly-known fisheries using hook-and-line methods in Brazil, to determine levels of incidental capture of seabirds and sea turtles, to identify potential impacts on endangered species and conservation needs.

METHODS

Study area

The study area stretches from 18°S to 35°S, corresponding to the fishing grounds of the Itaipava fleet or vessels from other southern ports using Itaipava-like methods. The area also encompasses the fishing grounds for the pole-and-line fleet using live bait and targeting Skipjack Tuna (*Katsuwonus pelamis*), which departs from Rio Grande, Itajaí and Rio de Janeiro ports.

The Falklands (Malvinas) Current carries cool SubAntarctic waters northward and meets the warm waters of the Brazil Current flowing southward, forming the Subtropical Convergence between about 25°S and 45°S, a high productivity area that holds important fish stocks and considerable numbers of top predators (Odebrecht & Castello 2001). In southern Brazil the continental shelf is wide (Fig. 10.1), with unconsolidated substrates, suitable for demersal fisheries such as trawling and bottom gill netting. Southern Brazil holds the bulk of Brazilian fishing effort as in northern areas the continental shelf is narrow, with coral reefs and shallow banks, where warm and oligotrophic waters of the Brazil Current predominate (Fig. 10.1; Olavo *et al.* 2005).

Sampling methods and effort

Observers collected detailed descriptions of different fisheries, and data on incidental capture of seabirds and sea turtles, during 16 cruises. Additional data were obtained through interview with captains, crew and from the literature, in order to characterize variations and vulnerability of birds and turtles.

From 2001 to 2006, 15 cruises departed from ports of Itaipava, Cabo Frio, Santos, Itajaí and Rio Grande, covering the range of different commercial fisheries using hook-and-line. One cruise was assessed by logbook provided by the fishing master and validated through five other cruises with the same vessel and crew. Validating was performed by checking for the species reported in the logbook and by contacting the fishing master in the subsequent trips, which made it possible to confirm the accuracy of data provided. A total of 178 fishing days were sampled covering the range of fisheries described here,

with sampling effort per fishery varying from 20 to 48 fishing days (Table 10.1). For the pole-and-line fishery using live-bait and targeting Skipjack Tuna, data on potential interactions with seabirds were assessed by observation of activities close to the vessel carrying the observer, as several vessels would fish around a moored buoy. In several cruises different fishing methods were used simultaneously or in different periods.

Fishing effort by surface longline for Dolphinfish and pelagic longline for Swordfish was expressed as number of hooks, and capture rate calculated as birds/1000 hooks or turtles/1000 hooks. Fishing effort for all fisheries was also presented as 'fishing day per vessel', and bycatch rate reported as birds/fishing day or turtles/fishing day allowing comparison of impacts among fisheries.

RESULTS

Fishery description

Fisheries were defined according to parameters such as gear, target species, fishing operation, season, areas, as well as their potential threat to seabirds and sea turtles. According to these criteria, seven hook-and-line fisheries were described, as below.

Fast trolling

Trolling fishery, locally known as '*corrico*' is a technique in which lines are trailed from the stern of a boat at different speeds. Lines are usually thick (2.5 mm) with variable length (5 m to 90 m) baited with squid, sardines, skin and meat of Skipjack Tuna, fresh pork skin or artificial lures such as strips of white rubber. Hooks are around 11 cm in total length, 'J' type, with flattened eye and barbed, similar to the Mustad® No. 2 'general purpose sea hook'. Length of the line and vessel speed are adjusted according to target fish: lines 5-12 m long and 3 knots for Bigeye Tuna, a fishery described below, and 70-90 m long and 7 knots for large Yellowfin Tuna *Thunnus albacares*, Albacore *T. alalunga*, and Dolphinfish. The hook is trailed on or close to the sea surface and a fisherman holds the line by hand. The fleet operating at Espírito Santo and Rio de Janeiro coasts, which includes the important Campos Basin fishing ground (Fig. 10.1), departs from Itaipava and Vitória ports. Target species are the Dolphinfish and tunas.

Fishing operations frequently occur close to oilrigs, moored or floating buoys or other objects. When close to fish aggregating devices (FADs), trolling is frequently used in alternation with handlining: the boat trolls from a given location to the fishing point close to the FAD, when the boat is kept drifting and handlines deployed; after drifting a distance of a few hundred meters, troll lines are deployed and the boat moves again to the fishing point.

Slow trolling

Slow trolling is a derivation of the above fishery, basically differing in speed of the vessel, in using the vessel as a FAD, and targeting mainly Bigeye Tuna (Schroeder & Castello 2007). Its impact on seabirds is consistently different (Table 10.1) and management also requires a different approach. Simultaneous with slow trolling, pole-and-line gear are used as a secondary fishing method, and artificial bait (white plastic tube) is attached to the large hook.

Handlining

For the handlining fishery, each fisherman deploys a thin line against the current (1.2-1.4 mm) and the hook is around 6 cm in total length, 'J' type, similar to the Mustad® No. 7 'general purpose sea hook'; or the 'Japanese type' hook, which is around 6 cm in total length, rounded, with a ring at the eye and point not curved. Hooks are baited with squid, sardines, and Skipjack or small tunas' meat. A few sardines or guts of tuna are released at the same time in order to attract the targeted Yellowfin and Albacore Tunas associated with FADs. The boat sails against the current and the engine is turned off close to the FAD, lines and hooks released and the fishing takes place while the boat drifts a few hundred meters away from the FAD. Frequently, the boat returns to the point close to the FAD trolling for tuna, as described above. Live baits kept onboard (e.g. Rough scads *Trachurus lathami*, Mackerel *Scomber* spp., Brazilian sardines *Sardinella brasiliensis*, squids or small tunas and Skipjack up to 20 cm in length) are also used. While the boat is drifting, frequently the hook remains close to the surface several meters away from the vessels, due to a small swivel, which makes the chumming and hooks available for seabirds to scavenge. Fishermen try to avoid birds taking the hook, pulling the line when birds are nearby. Fishing grounds are along the Brazilian continental shelf and shelf break, but oilrigs in the north and moored buoys in the south are preferred areas (Fig. 10.1).

Surface longline for Dolphinfish

The gear consists of a multifilament 5 mm mainline up to 5.2 nautical miles long, two secondary lines between small styrofoam buoys, and hooks around 5 cm in total length, 'J' type, similar to the Mustad® No. 8 'general purpose sea hook', baited with frozen Brazilian sardines, Skipjack meat or live bait (mackerel, or sardines). Secondary lines are 2 m long and hooks remain at 2-2.5 m from the surface (R. Dallagnolo, UNIVALI, *unpubl. data*). Itaipava fishermen developed this technique and it has spread to southern ports, with significant landings in Itajaí port (UNIVALI 2004). It is a strongly seasonal fishery, in November and December in southern Brazil in waters 200 m depth, and from October to February off Rio de Janeiro and Espírito Santo coasts (Martins & Doxsey 2006). Once or twice a day 600 to 1,200 hooks are deployed for around 4 h, and the boat sails along the mainline, hauling caught fish and rebaiting hooks. This fishery is sometimes carried out

during daytime, while the longline for Swordfish is carried at night. In the present study we sampled surface longline sets in both southern and northern fishing grounds (Fig. 10.2).

Pelagic longline for Swordfish

Detailed descriptions of the technique and gear used in the pelagic longline fishery for Swordfish are available from around the world (e.g. Brothers *et al.* 1999) and also in the SW Atlantic (Neves *et al.* 2006a). However, the fleet based in Itaipava deploys a shorter mainline (12 to 18 nautical miles) and lower number of hooks (800-1000) due to the small size of vessels. Their potential impacts on seabirds and sea turtles are thought to be high, as with traditional longlines.

Bottom dropline

The bottom dropline, locally named '*pargueira'*, is an artisanal gear with some variations, used to target large fish over rocks, sea mountains, coral reefs, or steep banks, up to 300 m deep. After a shoal is found by echo sounder, fishermen deploy the gear attaching the extremity to the vessel or holding it by hand. Dropline consists of a line 60 to 400 m long, with a swivel close to a stone or other weight (5 kg) used to keep the gear on the bottom. From the stone runs another main line 30 to 400 m long to which are attached 5 to 100 short secondary lines (0.4 m long) with hooks 5 cm of total length, 'J' type, flattened, similar to the Mustad® No. 8 or No. 9 'general purpose sea hook'. A distance of 30 cm separates secondary lines and at the end of the line another stone (10 kg) is attached. Basically, there are three variations of the fishery, from 10 to 100 hooks: a 'hand dropline' operated by several fishermen from the side of the vessel, the 'small boat dropline' or '*mar novo*' in which a mother vessel releases 8 to 22 small glass fibre boats, operated by one or two fishermen around the mother vessel (Costa *et al.* 2005; Martins *et al.* 2005); and the 'big dropline', which is attached to a buoy and flag while the mother vessel release 5 to 10 droplines. The Itaipava fleet operates from southern Bahia to Santa Catarina (Fig. 10.2), in depths from 40 m to 300 m, and the main target species are Snappers (*Ocyurus chrysurus*, *Lutjanus* spp., *Rhomboplites aurorubens*), Wreckfish (*Polyprion americanus*), Tilefish (*Lopholatilus vilarii*), Sandperch (*Pseudopercis munida*), Hakes (*Urophycis* spp.), and Groupers (*Epinephelus niveatus* and *E. marginatus*).

Pole-and-line with live bait

Under pole-and-line fishery we refer to industrial baitboat vessels targeting Skipjack Tuna attracted to the vessel using live bait and a 'shower-like' method, frequently close to moored buoys and used worldwide to catch tuna. It started in Brazil in 1979 and now operates mainly from Itajaí and Rio Grande ports, all year round, in an area that extends from 20°S to 35°S (Castello & Habiaga 1989; Meneses-de-Lima *et al.* 2000, Fig. 10.2).

Thirty-three vessels operate from the port of Itajaí (UNIVALI 2004), six vessels from Rio Grande, and a small number from Rio de Janeiro.

Seabird bycatch

A total of 47 albatrosses and petrels were captured in this study, 16 by slow trolling, 25 by handlining and 6 by surface longline for Dolphinfish (Table 10.1). Other fisheries did not capture seabirds, but pole-and-line also caused seabird injuries and mortality, as reported below.

The trolling fishery had a mean capture rate of 0.069 birds/day, but due to differences in methods the fast trolling for Yellowfin Tuna captured no seabirds, while the slow trolling for Bigeye Tuna captured all 16 albatross and petrels (0.410 ± 0.68 birds/day, Table 10.1). However, due to the large size of hooks, most birds were entangled in the line or hooked in the bill with only one Great Shearwater (*Puffinus gravis*) severely injured by external hooking.

Overall, handlining accounted for 25 seabirds captured (0.610 ± 1.45birds/day) with a mortality rate of 0.143 birds/day. In spite of a capture rate comparable to the slow trolling for Bigeye Tuna, the use of small hooks, which remain away from the vessel, caused six fatalities, i.e. birds were killed because they swallowed the hook.

In the surface longline fishery for Dolphinfish, four seabirds were caught and released alive and there were two fatalities, an overall rate of 0.147 birds/1000 hooks (or 0.15 birds/day). Due to small secondary lines and floating gear, baits remain available for seabirds during the whole fishing time, but this avoids drowning of hooked seabirds. For the pelagic longline for Swordfish, no seabird was caught, but number of hooks sampled was only 12,000 hooks.

In the bottom dropline sample, no incidental capture of seabird or sea turtle was recorded. The potential of this fishery for interaction with seabirds is low, but could cause a small bycatch of sea turtles, as reported by fishermen, or entanglement in the mainlines as reported in Uruguay (Laporta *et al.* 2006).

The fleet using live-bait to target Skipjack Tuna attracts large numbers of seabirds, mostly Cory´s Shearwaters (*Calonectris diomedea*), Cape Verde Shearwaters (*Calonectris edwardsii*) and Great Shearwaters (*Puffinus gravis*). Fishermen try to scare birds by hitting them with a metal piece attached to a pole-and-line. From a sample of 30 Great Shearwaters trapped at sea for another project, five birds (17%) had severe injuries (broken legs and scars on the back, neck and head). Injuries reported here were underestimated because they do not include lethal ones. In addition, at least four dead

Shearwaters (Great Shearwater and unidentified *Calonectris*) were observed floating on the sea surface in a single day in February 2006, probably killed in this way, as they were near three pole-and-line and ten handlining/trolling vessels fishing close to a moored buoy.

Sea turtle bycatch

Sampled fisheries captured 45 sea turtles: 44 by surface longline for Dolphinfish and one by pelagic longline for Swordfish. Other fisheries described here did not capture turtles. Fishermen reported occasional capture of turtles by bottom dropline, but these fisheries probably cause minor impacts on turtles.

Forty-four sea turtles of four species were captured by the surface longline for Dolphinfish, a rate of 1.08 turtles/1000 hooks (1.10 ± 1.72 turtles/day). Capture rate was high for Loggerheads (0.516 turtles/1000 hooks) and Leatherbacks (0.343 turtles/1000 hooks) and lower for Green and Olive Ridley, *Lepidochelys olivacea*. Regarding Loggerheads, eleven were caught entangled in the main and secondary lines or hooked externally, while seven swallowed the hook. Only one Loggerhead was captured dead. Curved Carapace Length (CCL) of Loggerheads varied from 64 to 80 cm (mean 71.8 cm, n = 13). All Leatherbacks (n = 14) were entangled or externally hooked, and were large immatures or adults not hauled onboard, which precluded measurements. Green Turtles were juveniles with CCL varying from 36 to 52 cm (n = 5), also entangled or externally hooked. The only Olive Ridley caught swallowed the hook but was released alive (CCL = 59 cm).

For the pelagic longline fishery for Swordfish, one adult Leatherback Turtle (CCL = 131 cm) was entangled and released alive. This gear was deployed in four trips, 30 sets and a total of 11,974 hooks. In two "Swordfish trips" the surface longline for Dolphinfish was also deployed, and captured sea turtles.

DISCUSSION

Fisheries and bycatch of seabirds and sea turtles

The Itaipava fleet operated several different hook-and-line methods depending on fishing grounds, target and season in a large area over the continental shelf and offshore waters, from 18°S to 35°S. Fishing grounds overlap with foraging areas of wintering and breeding albatrosses and petrels, as well as with sea turtles. This fleet is composed of 497 vessels, five times bigger than the whole national and leased pelagic longline fleet targeting tuna and Swordfish (89 vessels in 2005, Travassos and Hazin - Brazilian unpubl. report to ICCAT) that was previously recognized as the main threat for seabirds and sea turtles in Brazil. The Itaipava fleet has little to no control from governmental authorities regarding vessel licence, fishing operating licences, landing statistics, and

management. Their activities had only recently being considered by scientists (e.g. Martins *et al.* 2005; Martins & Doxsey 2006) and a high potential of interaction with seabirds and sea turtles was confirmed in the present study.

The trolling method is used all around the world in fisheries targeting tuna, Salmon (*Salmo* spp.), Barracuda (*Sphyraena barracuda*) and others (Majkowski 2003), with incidental capture of seabirds reported. In the Mediterranean, Cooper *et al.* (2003) reported that small Maltese vessels undertaking trolling for Tuna, Bream (*Dentex dentex*) and other predatory fish killed 35 birds, of which 71% were Cory's Shearwaters. Unpublished information in several countries reported captures of shearwaters (*Puffinus carneipes* and *P. pacificus*), Yellow-nosed Albatrosses, Australian pelicans (*Pelecanus conspicillatus*) and Boobies (*Sula* sp.) either by taking hooks or by colliding with gear and becoming entangled. The technique and gear used in Brazil have some differences in comparison with trolling elsewhere, with minor implications for the incidental capture of seabirds when targeting Yellowfin Tuna, but with major concern when targeting Bigeye tuna (catch rate of 0.41 birds/day). Information presented here and from other regions suggests that seabird capture in this trolling occurs commonly and needs to be better studied, particularly when the vessels troll lines slowly.

Handlines are used to catch different species of tunas all around the Pacific Ocean, Indian Ocean, Red Sea, Mediterranean and Atlantic Ocean, frequently around FADs. Handlines are also reported to be a selective fishing method (Majkowski 2003), but we found high levels of incidental capture and mortality in Brazil. The catch rate reported here of 0.61 birds/day is high, particularly if taking into account that 497 vessels compose the Itaipava fleet and that endangered species are being killed, such as the Spectacled Petrel (*Procellaria conspicillata*) and the Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*) (Cuthbert *et al.* 2003b; Ryan *et al.* 2006). Mortality in this fishery is also high because they use small hooks which can easily be swallowed by birds.

Surface longline for Dolphinfish in Brazil had a high bycatch of seabirds (0.147 birds/1000 hooks) above the rate reported in the pelagic longline in Brazil of 0.09 birds/1000 hooks (Neves *et al.* 2006a). However, the traditional pelagic longline captures seabirds during winter months (Neves *et al.* 2006a), while the surface longline for Dolphinfish takes place during summer. In Brazil this gear is deployed considerably shallower than longline for Dolphinfish in Costa Rica, which sets at a depth up to 10 m (Swimmer *et al.* 2005). A range of characteristics including low depth, deployment during daylight hours, and use of small hooks make it particularly dangerous for seabirds by being available throughout fishing and not only during deployment as in the longline for Swordfish and tuna. Catch rate of sea turtles was also high in the surface longline for Dolphinfish (1.08 turtles/1000 hooks) comparable to rates reported in the longline fishery

for Swordfish in the SW Atlantic of 0.68 to 2.85 turtles/1000 hooks (Domingo *et al.* 2006). Sizes of Loggerheads and Leatherbacks were similar to specimens captured in traditional pelagic longline for Swordfish in Brazil and Uruguay, with immature Loggerhead and immature and adult Leatherback Turtles predominating (Kotas *et al.* 2004; Domingo *et al.* 2006). Dolphinfish fishery landings in Itajaí started in 2001 with 2.7 million hooks deployed from 2001-2004 (R. Dallagnolo, UNIVALI, unpubl. data).

The pelagic longline for Swordfish captured no birds during the present study, nor in another study in the Espírito Santo area (Olavo *et al.* 2005). However, both studies have low sampling effort and could miss rare stochastic events, as is the incidental capture of seabirds in longlines. Fishermen reported the capture of birds in this fishery and additional data are needed for a definite conclusion. On the other hand, one Leatherback Turtle was captured in spite of low number of hooks deployed, consistent with other reports of capture in longline in the area (Olavo *et al.* 2005 – catch rate 0.297 turtles/1000 hooks; Marcovaldi *et al.* 2006) and nesting grounds in nearby Espírito Santo beaches (Barata *et al.* 2004). Espírito Santo is also a major nesting area for Loggerhead Turtles in Brazil (Baptistote *et al.* 2003) a species captured in high numbers in the SW Atlantic (Domingo *et al.* 2006; Marcovaldi *et al.* 2006), which means that both species and the Itaipava fishing fleet overlap and have a high potential of interaction.

Conservation actions and fisheries management

The fishing methods described here and adopted by Itaipava fleet, in particular the handlining, surface longline for Dolphinfish and the pelagic longline for Swordfish have an important role in the decline of seabirds and sea turtles, previously attributed to other fisheries, in particular the use of pelagic and bottom longlines (Brothers *et al.* 1999; Domingo *et al.* 2006). Slow trolling for Bigeye Tuna also has high capture rates, but with minor impacts on seabirds because only a handful of vessels use this method. Management actions for the fishery and their impacts on target and bycatch species need to be controlled by regulatory agencies and there is a need for monitoring of the fleet. Currently, the Itaipava fleet is regulated by target fish abundance and inventive capacity of their fishermen to explore new areas and species, with inefficient regulation by government. An effective program of monitoring with onboard observers is important for the assessment of impacts on endangered sea turtles and seabirds and differential vulnerability according to gear variations, fishing methods and environmental variables.

Mitigation measures to avoid the incidental capture of seabirds are available for bottom and pelagic longline and include bird-scaring lines, line setting at night, and dying baits (Brothers *et al.* 1999). For the pelagic longline for Swordfish described here bird-scaring lines and night setting should be effective. For the slow trolling for Bigeye Tuna and the

handlining for Yellowfin Tuna, scaring lines would probably work, but their effectiveness and impacts on target species catches need to be addressed.

For the surface longline for Dolphinfish, the major concern reported in the present study, alternative measures could be practical such as the deployment of weights, weighted line, or longer secondary lines with large swivels taking hooks below the surface. Longline gear used in Costa Rica, Pacific Ocean, targeting Dolphinfish and Tunas is deployed deeper (Swimmer *et al.* 2005) and could also be effective in Brazil. Blue-dying baits probably will be a poor mitigation measure because the bait used is sardine or Skipjack meat and not squid, and also was not effective in avoiding sea turtle capture (Swimmer *et al.* 2005). For the mitigation of the capture of sea turtles, no effective measure is obvious, particularly because the bulk of sea turtles and Leatherback Turtles are captured by entanglement. The improvement of handling procedures for sea turtles and seabirds, improving after release survival is required and could be attainable by educational campaigns and distribution of line-cutters and dehookers. Finally, if mitigations in fisheries do not prove effective, drastic actions are encouraged such as banning the fishing methods (e.g. surface longline for Dolphinfish) and establishment of area closures. No measure is expected to be effective in Brazil without continuous monitoring and strong enforcement, as is also the case in other countries such as artisanal fisheries capturing sea turtles in Mexico (Koch *et al.* 2006). Conservation of declining seabirds and sea turtles require urgent measures also focusing on poorly known fleets and fishing methods, which have not received attention around the world, but could be a significant mortality factor in several places.

Table 10.1. Summary of sampling effort for each fishery using hook-and-line in Brazil, seabirds and sea turtles caught and capture rates. --- Not applicable.

Fishery	Effort	No. Seabirds (Capture rates ± SD)	No. Turtles (Capture rates ± SD)	Seabird Species	Turtle Species
Fast Trolling	48 days	0	0	---	---
Slow Trolling for Bigeye	39 days	16 (0.41 ± 0.68 birds/day, range: 0-2)	0	7 BBA, 4 GS, 3 SP, 1 AYNA, 1 WCP	---
Handlining	41 days	25 (0.61 ± 1.45 birds/day, range: 0-7)	0	11 SP, 8 GS, 6 AYNA	---
Surface Longline for Dolphinfish	40 days & 40,717 hooks	6 (0.15 ± 0.58 birds/day, range:0-3 & 0.15 birds/1000 hooks)	44 (1.10 ± 1.72 turtles/day, range:0-8 & 1.08 turtles/1000 hooks)	2 AYNA, 2 *Thalassarche* sp., 1 MS, 1 WCP	21 LH, 14 LB, 8 GT, 1 OR
Pelagic Longline for Swordfish	31 days & 11,974 hooks	0	1 (0.032 ± 0.18 turtles/day, range: 0-1 & 0.08 turtles/1000 hooks)	---	1 LB
Bottom Dropline	20 days	0	0	---	---
Pole-and-line with live bait	41 days	0*	0	---	---

AYNA - Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*, BBA - Black-browed Albatross *Thalassarche melanophris*, GS - Great Shearwater *Puffinus gravis*, SP – Spectacled Petrel *Procellaria conspicillata*, WCP - White-chinned Petrel *Procellaria aequinoctialis*, MS – Manx Shearwater *Puffinus puffinus*, GT – Green Turtle *Chelonia mydas*, LH – Loggerhead Turtle *Caretta caretta*, LB – Leatherback Turtle *Dermochelys coriacea*, OR – Olive Ridley Turtle *Lepidochelys olivacea*. * Interactions with seabirds described in the text (floating dead birds, and 17% of live sampled Great Shearwater with injuries).

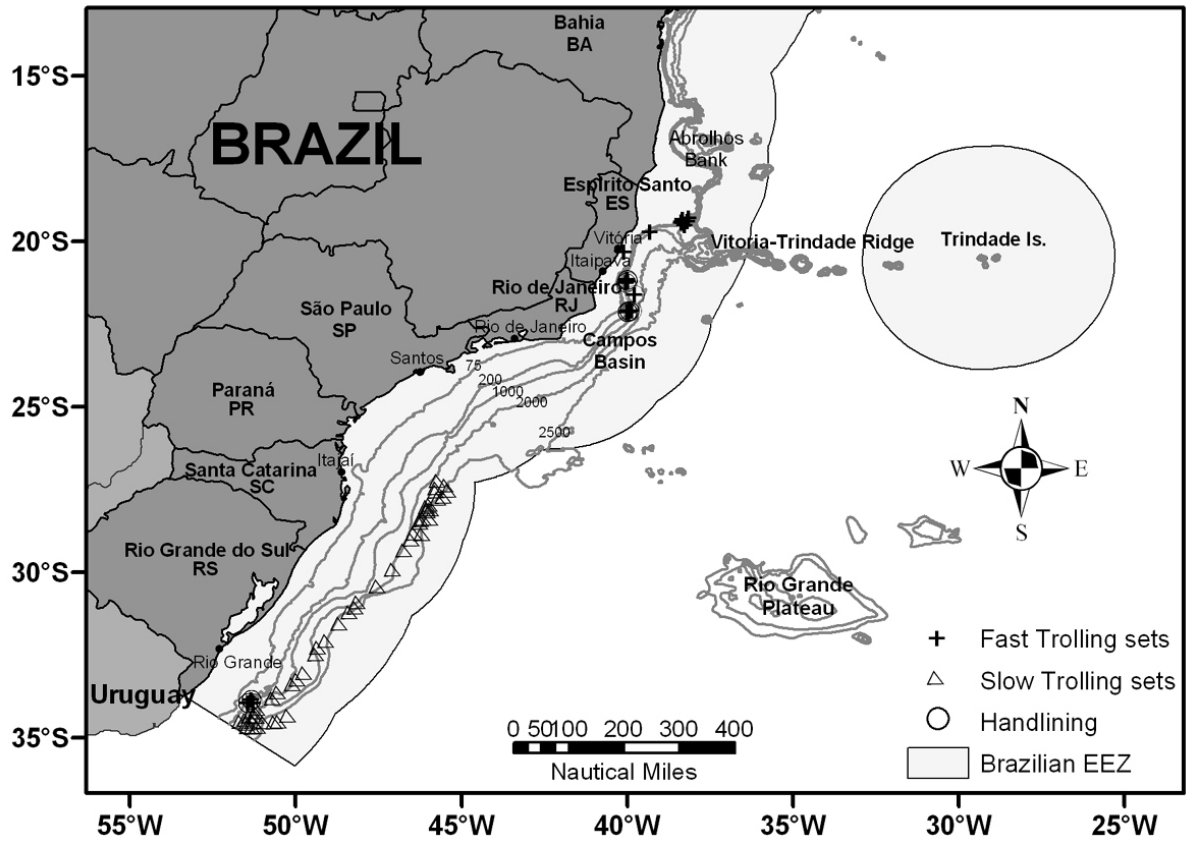

Figure 10.1. Slow and fast trolling fishery sets sampled from 2002 to 2006 in the SW Atlantic Ocean with Brazilian states, main fishing ports and Exclusive Economic Zones (EEZ) indicated. Handlining fishing grounds for tuna operated near oilrigs in northern areas and moored buoys in southern areas are also indicated.

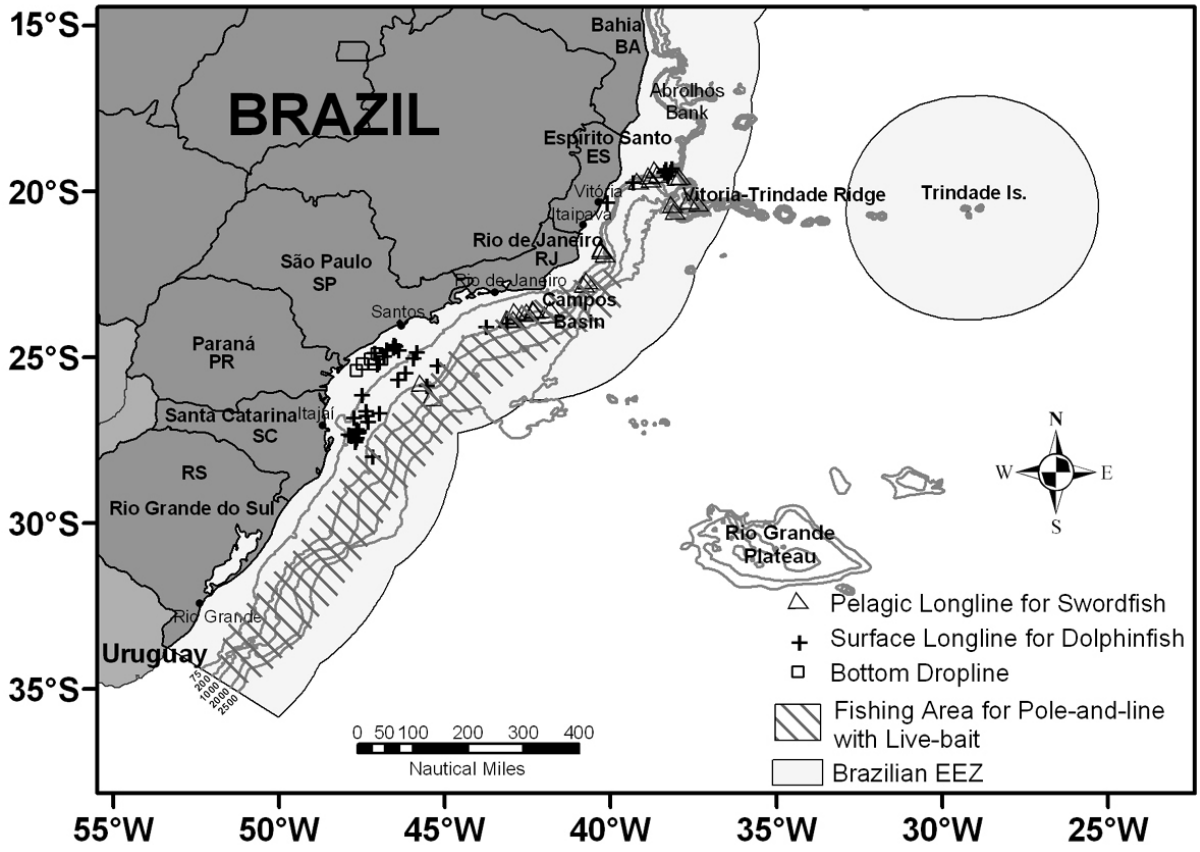

Figure 10.2. Sets of surface longline for Dolphinfish (*Coryphaena hippurus*), pelagic longline for Swordfish (*Xiphias gladius*), and Bottom Dropline sampled by onboard observers in Brazil from 2004 to 2006. Shaded area corresponds to the fishing grounds for the pole-and-line fishery using live bait and targeting Skipjack Tuna (*Katsuwonus pelamis*).

Chapter 11

SEX-BIASED INCIDENTAL MORTALITY OF ALBATROSSES AND PETRELS IN FISHERY: DIFFERENTIAL DISTRIBUTIONS AT SEA RATHER THAN SEXUAL SIZE DIMORPHISM

Bugoni L, Griffiths K, Furness RW. Sex-biased incidental mortality of albatrosses and petrels in fishery: differential distributions at sea rather than sexual size dimorphism. **Under review**

ABSTRACT

Skewed Adult Sex Ratio (ASR) has been proposed as a common pattern in birds, frequently biased towards males and with larger biases in globally threatened species. In albatrosses and petrels, differential mortality of one gender in fisheries is suggested to be caused by sexual size dimorphism giving males a competitive advantage, which allows more access of the larger sex (i.e. males) to discards and baits, or to at sea segregation of sexes. Here we tested these hypotheses by determining ASRs in albatrosses and petrels sampled at sea, and searched for patterns in ASR in albatrosses and petrels killed by fisheries and reported in the literature. We shown that skewed ASR is common in albatrosses and petrels from the community attending vessels, confirming results found for birds in general. However, there was no correlation between skewed ASR and conservation status, both in terms of population size or global level of threat, or between ASR and sexual size dimorphism. Skewed ASR toward males, females or parity is equally reported in the birds incidentally killed in fisheries. Thus, sexual dimorphism in size does not explain skewed ASR in the community we sampled or in incidental captures in fisheries reported in the review. Differential at sea distributions of males and females appear to explain better the patterns found in the community sampled at sea, as well as skewed ASR in seabird fatalities. Differential at sea distribution of Procellariiformes appears to contrast with patterns for other seabird taxa, where the sexes seem to share the same geographical range.

INTRODUCTION

Sex ratio in birds has received much attention from researchers, often studied at primary (at hatching) and secondary (at fledgling) levels, while tertiary level or adult sex ratio (ASR) as defined by Mayr (1939) is far less known (Donald 2007; Becker *et al.* 2008). In a recent review Donald (2007) emphasized that many questions posed by Mayr (1939) remain unanswered, and reported that skewed ASRs are common in wild bird populations (65% of studies), with males outnumbered females by around 33% on average despite offspring sex ratio not differing from parity. An emerging pattern from Donald's review was that globally threatened species are more prone to distortion in ASR, with profound implications for conservation. Furthermore, unbalanced ASR resulting from skewed mortality impacts on fecundity by reducing effective population size, an effect that persists longer after biased mortality ceases in albatrosses (Mills & Ryan 2005). In Wandering Albatross (*Diomedea exulans*) skewed primary and secondary sex ratios towards males were balanced at recruitment age due to higher mortality of immature males, but again biased towards males at adulthood by a slightly higher mortality of females (Weimerskirch *et al.* 2005). The higher adult mortality of females is associated with different at sea distribution, males occupying Antarctic waters (50-60°S) while females are mainly found in subtropical waters (35-45°S) and overlap more extensively with fisheries (Weimerskirch & Jouventin 1987; Croxall & Prince 1990).

Several studies have reported ASRs of albatrosses and petrels incidentally captured in fisheries with some reporting skewed ASR towards males (Ryan & Boix-Hinzen 1999; Nel *et al.* 2002b), some towards females (Bartle 1990), variable asymmetry towards both genders or not differing from parity (Gales *et al.* 1998). Reasons for biased sex ratios in fisheries fatalities have been suggested, but not specifically tested. Hypotheses for the discrepancies included different at sea distributions of males and females, and differential access to feeding resources (baits and discards) due to sexual size dimorphism, differential aggressiveness and manoeuvrability. In this study we determined the ASRs in a number of albatross and petrel species wintering off Brazil and sampled at sea. We related the ASR with their sexual size dimorphism. We hypothesized that if dimorphism plays a role in determining ASRs in captures, ASR will deviate more from parity in species with marked dimorphism, and be similar in closely related species. In addition we reviewed ASRs in Procellariiformes incidentally captured in fisheries and sampled by necropsies, and ASRs investigated on breeding grounds and assumed to be caused by differential mortality in fisheries. Our aim was to determine if there is a pattern in ASR towards males or females which could be used to understand the role of at sea segregation and size dimorphism. Finally, we tested the hypothesis of threatened species being more prone to asymmetry in sex ratio, as suggested recently (Donald 2007), by determining ASR in species with different levels of threats and different population sizes. Procellariiformes (albatrosses, petrels, shearwaters) are a particularly suitable group for testing these hypotheses because they are monogamous, extreme *k*-strategist species, i.e. long-lived with low fecundity and low adult mortality, occur in multi-specific flocks, and have several threatened and non-threatened species (Marchant & Higgins 1990; Tickell 2000; Brooke 2004).

METHODS

Study area and seabird sampling

Albatrosses and petrels were captured at sea when attending fishing vessels to scavenge on baits and waste. Vessels were targeting Tuna (*Thunnus* spp.), Sharks (mainly Blue Shark *Prionace glauca*) and Swordfish (*Xiphias gladius*) through a range of hook-and-line and pelagic longline fishing methods as described by Bugoni *et al.* (2008c). This fleet operates in deep waters over the continental shelf and in offshore waters in southern Brazil throughout the year (Mayer & Andrade 2005) in the region under the influence of the Subtropical Convergence, formed by the meeting of the warm tropical Brazil Current flowing southward and the cold Falkland (Malvinas) Current flowing northward (Garcia 1998). The overlap between fishing fleet and distribution of albatrosses and petrels is cause for concern due to incidental capture of these seabirds (Bugoni *et al.* 2008a, Chapter 9). We captured and sampled birds non-destructively during six cruises and 58 trapping days from February to June 2006 and August-September 2007, from 25 to

35ºS. Birds were attracted close to the vessel using baits and shark liver and captured using a cast net (Bugoni *et al.* 2008b). Wandering and Tristan (*Diomedea dabbenena*) Albatrosses are virtually identical in plumage (Onley & Scofield 2007), so we used the discriminant function in Cuthbert *et al.* (2003a) to assign species. We further confirmed identification after sexing birds using culmen length (see Cuthbert *et al.* 2003a for details).

Molecular sexing

Blood samples (1 mL) were taken by syringe and needle from the tarsal vein of every bird. Subsamples of blood were stored in 1.5 mL vials with absolute ethanol. Sexing of birds was carried out after DNA extraction and PCR amplification of CHD genes using primers 2550F (Fridolfsson & Ellegren 1999) and 2757R (R. Griffiths, umpublished) and genes separated in 2% agarose gel by electrophoresis. Briefly, copies of CHD genes are present in both Z and W bird sexual chromosomes. CHD-Z and CHD-W genes differ in the base pair length of their non-coding regions. Because females are heterogametic (ZW) and males homogametic (ZZ), the separation of gene amplification products by size results in a single band for males and two bands for females. DNA extraction negative controls were included for every 23 samples. Positive and negative controls were included for each PCR reaction. One third of all DNA extractions were repeated to confirm gender assignment. Furthermore, we used samples of previously sexed birds as controls: one male Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*) killed in fisheries and sex determined by necropsy, and four male and female Wandering Albatrosses of known sex (A. Wood and R.A. Phillips, British Antarctic Survey, *in litt.*).

Data analysis

ASR deviations from the expected proportion of 1:1 were tested by χ^2 test with Yates' correction for continuity due to only one degree of freedom (Fowler *et al.* 1998). Trapping methods of birds have been demonstrated to bias estimates of ASR (Domènech & Senar 1998). However, Procellariiformes studied here are sexually monomorphic in plumage, thus we were unable to detect visually any sex differences during captures and assumed that samples were representative of the population attending fishing vessels.

Sexual size dimorphism was calculated from biometric measurements of sexed birds. Detailed description of measuring procedure and values are presented elsewhere. The percentage of dimorphism between sexes in each trait was calculated as:

$$\left[(\bar{x}_m - \bar{x}_f)/\bar{x}_f\right]\times 100 \qquad \text{(equation 11.1)}$$

where $\bar{x}_m$ and $\bar{x}_f$ are mean values for males and females, respectively (Copello *et al.* 2006). We calculated dimorphism percentages for culmen, wing and tarsus lengths, and pooled the three percentages in a single dimorphism index for each species by calculating the average of values. Tail and body mass were not used because we found the former a poor descriptor of dimorphism (Chapter 6) and the later is very variable according to status of bird (Croxall 1995). Species included in the analyses attended fishing vessels for discards and most had been previously recorded to be incidentally captured in longline fisheries.

Conservation status follows categories proposed by IUCN (2007), with levels of threat, in increasing order as: least concern, near-threatened, vulnerable, endangered and critically endangered.

RESULTS

All birds from the 10 species included in this study, plus another four petrel species were successfully sexed using PCR methods, with no discrepancy between controls and replicates, confirming the reliability of the PCR-based method and primers for sexing Procellariiformes.

Six species had sample size large enough for statistical comparison. Three species (Atlantic Yellow-nosed Albatross, White-chinned Petrel *Procellaria aequinoctialis*, and Great Shearwater *Puffinus gravis*) has ASR not deviating from parity, while three species had biased ASR: Black-browed Albatross (*Thalassarche melanophris*) towards females, Cape Petrel (*Daption capense*) and Spectacled Petrel (*Procellaria conspicillata*) towards males. Wandering Albatross had five males and four females sampled, while Southern Fulmar (*Fulmarus glacialoides*) had two males and eight females and Southern Giant Petrels (*Macronectes giganteus*) had seven males and four females sampled. These species were not tested due to small sample size and were not included in further statistical analysis. ASR of Atlantic Petrel (*Pterodroma incerta*) sampled from a group of birds displaced inland after a hurricane (Bugoni *et al.* 2007) was also included. This species had ASR skewed towards females as determined by necropsies (Table 11.1).

Cape Petrel and Great Shearwaters are species not threatened by extinction, and only the former species had skewed ASR. Amongst the remaining species, two had even ASR, while four had skewed ASR: two towards females and two towards males. Pairs of threatened species phylogenetically closely related (*Thalassarche* albatrosses and *Procellaria* Petrels), show contrasting results: one species of each pair with skewed ASR.

Average sexual size dimorphism varied from 1.3% in Great Shearwater to 12.1% in Southern Giant Petrel, with larger males in all species (Table 11.2). The degree of sexual dimorphism was not correlated with the level of asymmetry in ASR (Spearman correlation coefficient r_s = 0.18, n = 11, *P* = 0.18). Similarly, the ASR was not correlated with population size (r_s = -0.29, n = 11, *P* = 0.53), and there was no clear pattern of ASR according to conservation status (Fig. 11.1).

Sixteen published studies reported ASR of eight albatrosses, four petrel and two shearwater species captured in fisheries (Table 11.3). One study reported more female Short-tailed Shearwaters (*Puffinus tenuirostris*) being captured in gillnets (DeGange & Day 1991) and the remaining studies refer to pelagic and bottom longline fisheries. Studies frequently tested for ASR in several species or carried out stratified analysis, by season and area. Overall, skewed ASR towards males was reported 19 times (39%), parity 18 (37%) and bias to females reported 12 times (24%). Skewed ASR in Procellariiformes was reported more frequently than parity (χ^2_{Yates} = 11.76, *P* =0.0006), but considering the three possible results (males, females and parity) there was no tendency towards any gender or parity to be reported more frequently (χ^2_2 = 1.76, *P* = 0.42). The most detailed study (Gales *et al.* 1998) tested for ASR in different areas and seasons and found that some species are skewed during one season or in one area but show even ASR in other areas or seasons, or a species could show biased ASR towards males, females and no bias according to season or area (Table 11.3).

DISCUSSION

The present study showed skewed ASR in a number of species of albatrosses and petrels belonging to a community at sea. However, our results failed to demonstrate an association between skewed ASR and conservation status, both in terms of population size or global level of threat, as suggested by Donald (2007). Determination of ASR may be subject to a number of biases (Donald 2007), but sampling and determining sex of a range of species occuring simultaneously in the same area using the same method appears to be adequate to test the hypothesis proposed by Donald (2007) and confront with alternative ones, such as differential distribution and sexual size dimorphism. Based on data obtained in the SW Atlantic and revision from the literature, we failed to find association between ASR and conservation status, which would be tested in other areas and taxa. However, if regional differences in sex-ratio are widespread in Procellariiformes and other birds, the relationship between biased sex-ratio and conservation status should be better analysed on a global scale rather than regionally, as frequently occurs.

Differential mortality of one gender is of serious conservation concern for endangered seabirds as albatrosses (Mills & Ryan 2005) of which 19 out of 21 species are threatened by extinction (BirdLife International 2004). However, sexual dimorphism in size do not

appears to explain differential access to discards and baits by males and females either in data in this study, or in our review of reported ASRs. There was no pattern of skewed mortality towards males in the range of ASR mortality reported in the literature, with parity, male bias and female bias being equally reported. The consistent male biased mortality of White-chinned Petrel, Gray-headed (*Thalassarche chrysostoma*) and Indian Yellow-nosed *Thalassarche carteri*) Albatrosses reported by Ryan & Boix-Hinzen (1999) and confirmed with a larger data set for these species plus Grey Petrel, *Procellaria cinerea* (Nel *et al.* 2002b) in longline fisheries around Prince Edward Islands, was not confirmed in other studies, suggesting that the larger size of males or higher male aggressiveness do not play an important role in seabird captures. The hierarchy in access to discards in seabird communities is based in body size (Barnes *et al.* 1997) and this is also clear in our study area, where the preferential access to feeding source is *Diomedea* > *Thalassarche/Macronectes* > *Procellaria* > *Puffinus* > *Daption*/*Fulmarus* (author's, pers. observ.). However, it is improbable that the small sexual size dimorphism within species we observed (Table 11.2) account for the variation in captures. Sexual size dimorphism also fails to explain why closely related species (White-chinned and Spectacled Petrels; Atlantic Yellow-nosed and Black-browed Albatrosses) could have even or skewed sex ratios, as well as fails to explain the female skewed ratios we found.

An alternative hypothesis is differential at sea distribution of males and females. Sexual segregation has been clearly demonstrated in some Procellariiformes, such as Southern Giant Petrel and Northern Giant Petrel (*Macronectes halli*) (González-Solís *et al.* 2008), Black-browed and Gray-headed Albatrosses (Phillips *et al.* 2004b), Southern buller's Albatross (*Thalassarche bulleri*) at the Snares Island (Stahl & Sagar 2000a), and suggested in other studies (Bartle 2000; Shaffer *et al.* 2001; Weimerskirch *et al.* 2005). Different at sea distribution has been argued as responsible for sex biases in albatrosses and petrels washed ashore (Holmes 1981) or driven inland by storm (Bugoni *et al.* 2007). Shaffer *et al.* (2001) showed that wing loading is lower in adult females and fledgling Wandering Albatrosses, and suggested that it makes them better adapted to exploit lighter winds of the subtropical and tropical regions. Further, Croxall & Prince (1990) reported more females being killed by tuna longliners in the SW Atlantic Ocean. However, males and females, juveniles and breeding adults of Wandering and Tristan Albatrosses were captured in the subtropical SW Atlantic Ocean in similar proportions in this study. Similarly, Dénes & Silveira (2007) and Dénes *et al.* (2007) reported nine females and four males Tristan, and seven females and two male Wandering Albatrosses specimens in Brazilian museums of birds stranded on the coast or captured in fisheries. Thus, these data pooled with data we obtained (Table 11.1) do not show a clear pattern of subtropical waters in the SW Atlantic being occupied preferentially by females. Similarly, Wandering Albatrosses attending fishing vessels in Falkland (Malvinas) and Scotia Ridge waters had sex ratio not differing from the expected 1:1, and all age classes

were recorded (Otley *et al.* 2007a). Latitudinal at sea segregation is not clear for Wandering Albatrosses in Atlantic Ocean and even less is known for most albatross and petrel species in the area. In addition, sex segregation at sea was not demonstrated in some species or populations (e.g. Anderson *et al.* 1997; Stahl & Sagar 2000b). Studies on other seabirds (gulls, penguins, cormorants) suggest that males and females share foraging areas, whereas have niche segregated in other dimensions such as foraging depth and prey size (e.g. Wernham *et al.* 2002; Phillips *et al.* 2004b and references therein). Albatrosses, petrels and shearwaters disperse over vast areas far from breeding grounds, which could play a role in explaining sexual and age-related at-sea segregation, both by competitive exclusion or habitat specialization. Differential distribution could be an important factor in skewed ASR in albatross and petrel communities and in samples of birds captured in longline fisheries. Moreover, age-related differences in at-sea distribution and in capture rates are a common pattern in several places, including the studied area. For example, Black-browed Albatrosses and White-chinned petrels, the main species captured in pelagic longline off Brazilian coast (Bugoni *et al.* 2008d, Chapter 9) are mainly juveniles, while Spectacled Petrels in the same area are adults (Chapter 6). Juveniles are more susceptible to captures in fisheries (e.g. Neves & Olmos 1997).

Benito & González-Solís (2007) suggested that adjustment in sex ratios in birds could occur during growth, i.e. from hatching to fledging, and Becker *et al.* (2008) demonstrated that sex ratio in Common terns (*Sterna hirundo*) varies from predominantly females at fledging to predominantly males at recruitment, through a mechanism of immigration of females and high natal phylopatry of males. In Wandering Albatross, Weimerskirch *et al.* (2005) show that male skewed sex ratio is present from birth, despite higher mortality of males during immature ages, which is sufficient to compensate for the biased sex ratio at recruitment. But due to slightly higher adult female mortality, there is skewed ASR toward males after 23 years old. Awkerman *et al.* (2006) found that intentional mortality by fishermen and incidental capture in longline and gillnet fisheries of Waved Albatross (*Phoebastria irrorata*) off Peruvian coast is predominantly of males (82%), which leads to female biased ratio at adulthood, despite even ratios at hatching, fledging and recruitment ages (Awkerman *et al.* 2007).

Due to the effects caused by asymmetrical mortality on endangered populations by reducing effective population size, the topic deserves further studies in a species-specific basis. It is also essential that fisheries observers retain seabirds killed in fishing operations and return carcasses for analysis. Sexual segregation of albatrosses and petrels at sea appears to be a widespread but poorly documented pattern, which could explain most differential captures in fisheries.

Table 11.1. Sex ratio and status of conservation of albatrosses and petrels sampled at sea in 2006 and 2007 in the southwestern Atlantic Ocean. For all tests df = 1. -- Sample sizes do not allow statistical testing.

Species	Males: Females	% of Males	χ^2 Yates	*P* values	Conservation Status	No. of breeding pairs
Puffinus gravis	29:38	43	0.96	*P* = 0.33	Least Concern	6,000,000[a]
Daption capense	22:9	71	4.65	*P* = 0.03	Least Concern	~1,000,000[b]
Fulmarus glacialoides	2:8	20	--	--	Least Concern	400,000[c]
Macronectes giganteus	7:4	64	--	--	Near Threatened	31,360[d]
Procellaria aequinoctialis	20:10	67	2.70	*P* = 0.1	Vulnerable	2,500,000[b]
Procellaria conspicillata	46:18	72	11.39	*P* = 0.0007	Vulnerable	>10,000[e]
Diomedea exulans	5:4	56	--	--	Vulnerable	14,000[f]
Pterodroma incerta[1]	6:23	21	8.83	*P* = 0.003	Endangered	1,800,000[g]
Thalassarche melanophris	6:27	18	12.12	*P* = 0.0005	Endangered	530,000[h]
Thalassarche chlororhynchos	21:12	64	1.94	*P* = 0.16	Endangered	36,800[a]
Diomedea dabbenena	3:1	75	--	--	Critically Endangered	2,400[i]

[1]Data from Bugoni *et al.* (2007). Population estimates: [a]Brooke (2004); [b]BirdLife International (2007); [c]Creuwells *et al.* (2007); [d]Patterson *et al.* (2006) in Quintana *et al.* (2006); [e]Ryan *et al.* (2006); [f]Croxall & Gales (1997); [g]Cuthbert (2004); [h]Lawton *et al.* (2003); [i]Cuthbert *et al.* (2004).

Table 11.2. Percentage of sexual size dimorphism (SSD) in external body measurements of albatrosses and petrels sampled at sea off Brazil. NA – not available.

Species	Culmen	Tarsus	Wing	Average SSD %
Diomedea dabbenena	6.3	0.8	NA	3.8
Diomedea exulans	4.8	7.8	6.4	6.3
Thalassarche melanophris	0.5	3.0	3.5	2.3
Thalassarche chlororhynchos	4.5	5.5	2.0	4.0
Macronectes giganteus	15.2	12.3	8.9	12.1
Fulmarus glacialoides	6.1	7.3	5.5	6.3
Daption capense	3.3	4.0	1.8	3.0
Procellaria aequinoctialis	3.6	1.2	1.2	2.0
Procellaria conspicillata	6.1	3.1	3.0	4.1
Puffinus gravis	2.6	1.4	-0.15	1.3
Pterodroma incerta	3.1	1.7	0.6	1.8

Table 11.3. Review of adult sex ratios of albatrosses and petrels incidentally captured in fisheries. The number of times a given result was reported (not skewed or towards each gender) is given.

Species	Skewed towards	Fishery Type	Reference
Grey Petrel (*Procellaria cinerea*)	Female	Pelagic longline	Bartle (1990)
Wandering Albatross (*Diomedea exulans*)	Female (ring recoveries)	Attributed to Pelagic longline	Croxall & Prince (1990)
Grey Petrel	Female	Pelagic longline	Murray *et al.* (1993)
White-chinned Petrel (*Procellaria aequinoctialis*)	Males (but not significant)	Bottom longline	Barnes *et al.* (1997)
Wandering Albatross	Females (skewed towards males in colonies)	Attributed to trawlers (colonies)	Weimerskirch & Jouventin (1987) and Weimerskirch *et al.* (2005)
Black-browed (*Thalassarche melanophris*) and Atlantic Yellow-nosed (*T. chororhynchos*) Albatrosses	Female (both species)	Pelagic longline	Calculated from data in Neves & Olmos (1997)
Black-browed (*Thalassarche melanophris*) and Campbell (*T. impavida*) Albatrosses pooled	*Not skewed (3x) Males (3x)	Pelagic longline	Gales *et al.* (1998)
Indian Yellow-nosed Albatross (*Thalassarche carteri*)	Not skewed (2x) Male (1x) Female (1x)	Pelagic longline	Gales *et al.* (1998)
Flesh-footed Shearwater(*Puffinus carneipes*)	Females (3x) Not skewed (1x)	Pelagic longline (tuna)	Gales *et al.* (1998)
Gray-headed Albatross (*Thalassarche chrysostoma*)	Males (2x) Not skewed (2x)	Pelagic longline	Gales *et al.* (1998)
Wandering Albatross	Males (3x) Not skewed (1x)	Pelagic longline	Gales *et al.* (1998)

Table 11.3. Continued

Grey Petrel	Not skewed (2x)	Pelagic longline	Gales *et al.* (1998)
White-chinned Petrel	Females (1x)	Pelagic longline	Gales *et al.* (1998)
Shy Albatross (*Thalassarche cauta cauta*)	Not skewed (3x) Females (1x)	Pelagic longline	Gales *et al.* (1998)
White-chinned Petrel, Gray-headed and Indian Yellow-nosed Albatross	Males (all three species)	Bottom longline	Ryan & Boix-Hinzen (1999)
Gray-headed and Indian Yellow-nosed Albatrosses, White-chinned and Grey Petrels	Males (all four species)	Bottom longline	Nel *et al.* (2002b)
Giant Petrels (*Macronectes giganteus/halli*)	Not skewed	Bottom longline	Nel *et al.* (2002b)
Black-browed Albatross	Not skewed	Bottom longline	Reid *et al.* (2004)
White-chinned Petrel	Male	Bottom longline	Delord *et al.* (2005)
Waved/Galápagos Albatross (*Phoebastria irrorata*)	Male	Gillnet and Longline fisheries, and intentional capture	Awkerman *et al.* (2006)
Black-browed Albatross and White-chinned Petrel	Not skewed	Bottom longline	Gandini & Frere (2006)
Black-browed Albatross and White-chinned Petrel	Not skewed	Bottom longline	Seco-Pon *et al.* (2007)
Flesh-footed Shearwater	Male	Pelagic longline	Calculated from data in Thalmann *et al.* (2007)
Short-tailed Shearwater (*Puffinus tenuirostris*)	Female	Gillnet	DeGange & Day (1991)

* Gales *et al.* (1998) presented sex ratios for season and area.

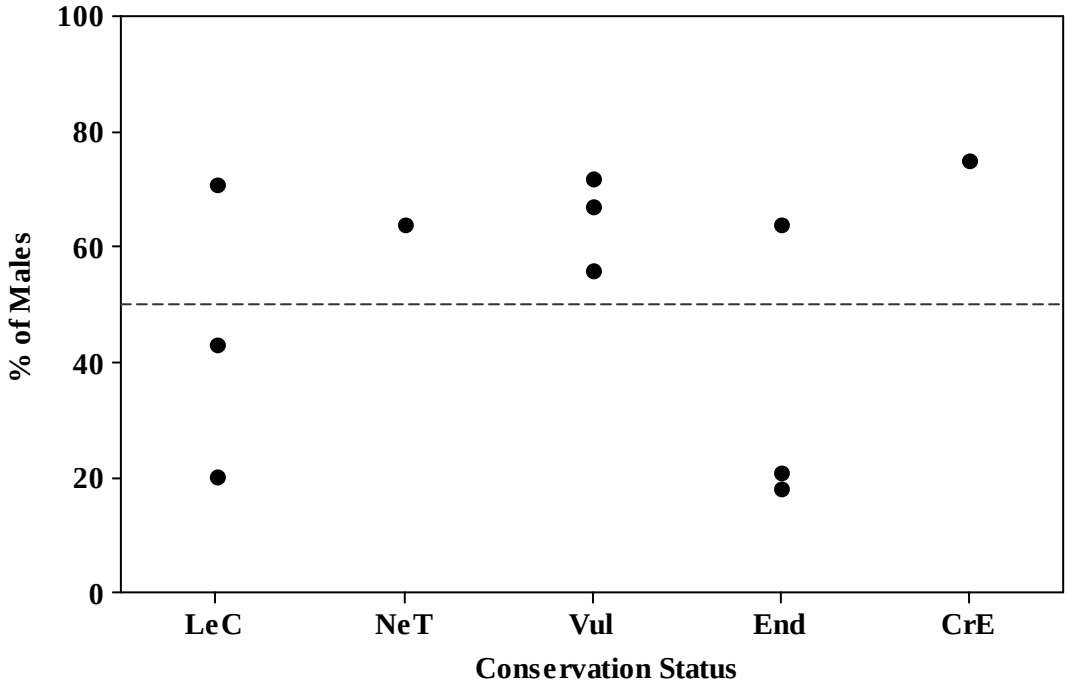

Figure 11.1. Adult sex ratio given as % of males of albatross and petrel species sampled at sea off Brazil, according to IUCN conservation status. LeC – Least Concern; NeT – Near Threatened; Vul – Vulnerable; End – Endangered; CrE – Critically Endangered.

Chapter 12

GENERAL DISCUSSION

This study was based primarily on albatrosses and petrel sampled at sea off southern Brazil or data from incidental capture in fisheries. Other activities were carried out during the last two years and data collected from these were intended to be included, but mainly due to time limitations this material is not part of the thesis and results briefly mentioned in Appendix 3 with cautionary notes on field work limitations as guidance for future studies.

Outlook on Procellariiformes of Brazil: Have we moved forward?

It is not my intention to provide here a discussion of the thesis, repeating the conclusions and main findings, which I hope was satisfactorily addressed in each previous chapter. However, it is useful to mention what was learnt from this study and how it could help in conservation.

Overall, it has been demonstrated that sampling seabirds at sea non-destructively is feasible and potentially applicable elsewhere in a range of biological and conservation studies. It could increase considerably our understanding on seabird life-histories and ecology during the long period when they are far from colonies. It provided us with data otherwise unavailable, such as on first year juveniles, older immatures, and individuals in sabbatical year, and allowed us to address questions on comparative moult strategies during active moult periods, proposed ageing methods, deploy transmitter on wintering birds, investigate sex ratio and sexual size dimorphism, and the trophic structure of the wintering community. As any other methods, sampling at sea has limitations, but complements the vast information available to date on breeding colonies, and overcome drawbacks on information obtained from beached birds, from remote sensing devices or other methods. Integrating a range of methodologies, both traditional and novel ones, with data from fisheries, abiotic data and incidental capture represents a step forward for a more complete understanding of seabirds at sea and for conservation of endangered albatrosses and petrels. From a Brazilian perspective, we moved from a scenario of studies restricted to occasional records of species or biased sampling of birds washed ashore to an ecological and wide-scale approach. For the first time in Brazil satellite transmitters were used to track seabirds and for the first time stable isotopes and geolocators were applied in bird studies. A critical review on incidental captures was undertaken and intended to provide insights for further studies, and additional threats from other fisheries were described.

The present study determined patterns of the offshore seabird community in terms of age and sex, determined which species and when they are incidentally captured in different fisheries. Through stable isotopes of carbon and nitrogen was demonstrated the surprisingly high dependence of most seabird species on fishing discards provided by pelagic longline. Despite a much clear panorama on seabird ecology in the area, several

questions still remain to be addressed. The main questions are probably those related to origin of seabirds, particularly those breeding in several different places, such as Cape Petrel, Southern Fulmar, Wilson's Storm-petrel, and Southern Giant Petrel. The origin of species could be addressed through analysis of trace elements in feathers, molecular markers or tracking birds using remote sensing, particularly satellite and GPS-satellite transmitters. All methods have some advantages and limitations according to species and associated economic costs. Species of particular interest for tracking are the Atlantic Yellow-nosed Albatross and additional tracking of Spectacled Petrels during the breeding season to determine if birds breeding in Tristan da Cunha Islands forage near the South American coast. The role of other fisheries in providing discards for seabirds should also be addressed through observations onboard vessels and stable isotopes, particularly the large trawling fleet operating in southern Brazil. Other topics not addressed here could be of ecological and conservation interest, for example the analysis of organochlorine residues and heavy metals in tissues of birds, how pollutant burdens vary in relation to trophic level, species, age and sex. This study is relatively easy to be done through sampling of live birds captured at sea as demonstrated here, and also could make use of carcasses obtained from fatalities in fisheries.

As explained in several previous chapters, I believe that the method of sampling birds attending longline vessels through a cast net provides a representative sampling of species and individuals in the area. In terms of species, just a few species not attending vessels for discards were not sampled, such as Prions (*Pachyptila* spp.), Cape Verde Shearwater (*Calonetris edwardsii*) and Atlantic Petrel (*Pterodroma incerta*), but all frequent and abundant species were sampled in proportions similar to their abundance and provided a biological reliable sample size. In terms of individuals, it is tempting to assume that sampling was representative, due to the high mobility of all Procellariiformes investigated and because they stay for short periods attending the same vessel. Thus, they could easily commute between different vessels and different fisheries, or between discards from vessels and more 'natural' foods. However, the possibility that some individuals are specialized in discards whereas others feed on natural food sources could not be ruled out. Further studies on individual specialization are necessary, and sampling the same species associated with other fisheries could add some light on this subject. Based on information currently available, it seems reasonable to assume that sampling for this study was representative, if not as a true sampling of the whole community, at least a genuine sampling of the 'community of seabirds attending pelagic longline vessels'.

In summary, I consider that this study provides a better understanding of the general biology and ecology of Brazilian birds from a different perspective and with important implications for conservation and management. Through the description of alternative

methods for studying seabirds in wintering areas I hope to stimulate additional studies on other aspects of the seabird ecology in Brazil and on wintering seabirds elsewhere.

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Appendix 1

Quillfeldt P, Bugoni L, McGill RAR, Masello JF, Furness RW. Differences in stable isotopes in blood and feathers of seabirds are consistent across species, age and latitude - Implications for food web studies. ***Under Review***

Differences in stable isotopes in blood and feathers of seabirds are consistent across species, age and latitude – implications for food web studies

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Abstract

Stable isotopes of growing feathers and blood both represent assimilated diet, and both tissues are used to study the diet and foraging distribution of marine and terrestrial birds. Although most studies have assumed that both tissues represent a difference of one trophic level to diet, the enrichment factors of blood and feathers may differ, especially where endogenous reserves are used as precursors during feather synthesis. In this study we compare carbon and nitrogen stable isotopes of blood and simultaneously growing feathers of five species of Procellariiformes, representing 5 genera, different geographical regions and different life stages (chicks and adults). In all species, feathers were enriched in ^{15}N and ^{13}C compared with blood. Isotopic values of carbon and nitrogen were correlated in different tissues growing simultaneously for most species analysed, suggesting that mathematical corrections could be used to compare different tissues. Our results imply that more care needs to be taken when comparing stable isotope signatures across studies assuming different tissues are equivalent indicators of trophic ecology.

Introduction

Recent developments in our knowledge about the natural distribution of stable isotopes in marine food webs have provided us with a useful tool to study movements and the trophic level at which seabirds feed, which can be applied when more direct studies are impossible (e.g. Cherel *et al.* 2005a, 2006, Quillfeldt *et al.* 2005, Bearhop *et al.* 2006, Gladbach *et al.* 2007). In particular, carbon stable isotope ratios can reflect foraging locations (reviewed in Rubenstein and Hobson 2004), while nitrogen isotope ratios become enriched in ^{15}N with trophic level by approximately 3.0 to 5.0‰ (Minagawa and

Wada 1984, Owens 1987) and $\delta^{15}N$ can thus indicate trophic position and be used to infer dietary composition (e.g. Hobson and Welch 1992, Hobson *et al.* 2000, Forero *et al.* 2005). Depending on the tissue chosen, dietary information spanning different temporal scales can also be obtained (Hobson and Clark 1992a).

The stable isotope approach is especially useful for studies of diet and foraging areas outside the breeding season, when pelagic birds are not usually accessible for sampling using traditional methods (Barrett *et al.* 2007). A number of studies have made use of the fact that the molting period is in this time, and thus, feathers grown by adult seabirds reflect the diet in the interbreeding period (e.g. Nisbet *et al.* 2002, Quillfeldt *et al.* 2005, Cherel *et al.* 2006, Gladbach *et al.* 2007). To assess diet and foraging areas during the breeding season, on the other hand, several tissues can be sampled non-destructively, including blood and feather samples of chicks and adults. However, due to the specific metabolic processes involved in tissue synthesis, different tissues may differ in their isotopic enrichment factor relative to the diet (e.g. Hobson and Clark 1992a).

As noted by several authors (Bearhop *et al.* 2000a, Cherel *et al.* 2005 a, b), differences in isotopic discrimination among tissues are a potential problem when comparing different tissue types. Temporal studies comparing the blood (representing the breeding season) and feathers (representing the interbreeding season) of adult seabirds are dependent on the assumption that isotope analyses of the two tissues provide directly comparable dietary proxies. However, most researchers working in the field of isotope ecology are aware that the enrichment factors of blood and feathers may differ when endogenous reserves are used as precursors during feather synthesis (Cherel *et al.* 2005a) or when blood contains ^{15}N-depleted uric acid (Bearhop *et al.* 2000b). Thus, most studies have limited their analysis to either blood or feathers, but have assumed both tissues to represent a difference of one trophic level to diet. A few studies have directly compared feather and blood isotope values (e.g. Podlesak *et al.* 2005, Bearhop *et al.* 2006), mainly on the assumption that the influence of tissue specific fractionation is small compared to the effect they aimed to measure.

The aim of this study is to compare the carbon and nitrogen stable isotope values of blood and simultaneously growing feathers of five species of Procellariiformes in order to test for differences in isotopic tissue discrimination between tissues. We also hope that by testing the basis of assumptions that are often made in dietary studies, regarding the equivalence of different tissues, we can help clarify if blood or feather is the optimal tissue to sample. We also consider the limitations of extrapolating different-tissue stable isotope comparisons within temporal studies or between studies using feather and studies using blood.

Materials and methods

Study site, species and sampling

Species, abbreviations used in Fig. 1 and sampling locations are listed in Table 1, for sample sizes see Table 2.

Great shearwaters *Puffinus gravis*, Atlantic Yellow-nosed albatrosses *Thalassarche chlororhynchos* and Spectacled petrels *Procellaria conspicillata* were sampled in the Southwestern Atlantic Ocean off the Brazilian coast, onboard fishing vessels from February to June 2006. Birds scavenging discards were attracted to the vessel and trapped with a cast net as described by Bugoni *et al.* (2008b). Blood was collected from the tarsal vein using a 2 ml disposable syringe and needle. Growing body feathers, i.e. still with blood in the calamus, from the breast and dorsum were collected and stored dry in sealed plastic bags. This sample includes immature as well as breeding and non-breeding adults.

Trindade petrels *Pterodroma arminjoniana* were sampled in breeding colonies of Trindade Island (*c*. 20°30'S–29°19'W), where there is a population estimated at 1130 breeding pairs (Luigi *et al.* 2008). Blood and growing feathers were collected from December 2006 to April 2007 and preserved in the same way described above. Four adults were sampled during chick feeding stage, whereas 14 were prospecting birds, but both groups were sampled over the same time period despite differences in breeding stage as the species nests throughout the year (Luigi *et al.* 2008). During the prospecting period (i.e. before the pre-laying exodus, which lasts usually two months), adults are frequently in final moult stage, with some growing body feathers still present (Luigi *et al.* 2008). Chicks were sampled following the same procedure, and full grown or growing body feathers of the definitive plumage were collected. One chick was 40 days old and others were 70 to 100 days old, so close to fledging, which occurs about the 95th to 100th day (Luigi *et al.* 2008). The half-life of isotope turnover of avian whole blood has been determined to be 11.4 days for quail (Hobson and Clark 1992a), and so represents the same time window as growth of new body feathers, as these feathers are 20-30 mm long and growth rate of feathers in birds varies from 2.5 to 10 mm per day (Langston and Rohwer 1996).

Feather and blood samples from chicks of Thin-billed prions *Pachyptila belcheri* were collected as part of ongoing studies of their breeding biology at New Island Nature Reserve, Falkland Islands (e.g. Quillfeldt *et al.* 2003, 2006, 2007a, b, c). The present study includes samples collected from 40-day old chicks during the breeding seasons 2004-05 (N = 7) and 2005–06 (N = 10). Birds were captured by hand and blood samples (0.2-0.4 ml) were collected from the brachial vein using heparinised capillaries within 2 min from burrow opening to the end of blood sampling. Blood samples were immediately transferred to 0.5-ml tubes and kept on ice until centrifugation. Plasma (used for hormone analyses, e.g. Quillfeldt *et al.* 2006, 2007c) and blood cells (used in

the present analysis) were stored frozen in separate 0.5-ml tubes at −20°C. Undertail covert feathers of chicks were collected shortly before fledging by gentle pulling and placed in individual sealed plastic bags. The first tail feathers of chicks including the tail coverts start to emerge after 3 weeks of age (e.g. Strange 1980: "at 22 days the sheaths of the rectrices can be felt protruding"), therefore the distal parts of these feathers represent the middle of the nestling growth period of 50−56 days. The half-life of isotope turnover of avian red blood cells was 29.8 days in crows *Corvus brachyrhynchos* (Hobson and Clark 1993) and 10.9 days for Yellow-rumped warblers *Dendroica coronata* (Podlesak *et al.* 2005). The red blood cell samples collected from Thin-billed prion chicks therefore represented the diet ingested ca. 2−4 weeks before the sampling at 20 days, i.e. at the same time as the analysed feather parts.

Sample preparation and stable isotope analysis

Feathers were cut into small fragments using stainless steel scissors, and whole blood and red blood cells (RBC) were freeze-dried and ground. Carbon and nitrogen isotope analyses were carried out on 0.65−0.7 mg aliquots of homogenized powder, weighed into tin cups.

Carbon and nitrogen isotope ratios were measured simultaneously by continuous-flow isotope ratio mass spectrometry (CF-IRMS) using a Costech Elemental Analyser (EA) linked to a Thermo Finnigan Delta Plus XP Mass Spectrometer. Two laboratory standards were analysed for every 10 unknown samples, allowing any instrument drift over a typical 14 hour run to be corrected. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviation from the international standards V-Pee Dee belemnite (carbon) and AIR (nitrogen), according to the following equation $\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$ where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}N/ ^{14}N$ or $^{13}C / ^{12}C$. Measurement precision of both $\delta ^{15}N$ and $\delta ^{13}C$ was better than 0.3‰.

Data analysis

Statistical tests were performed in Sigma Stat 2.03. All tests used pairwise data for each individual, carried out separately for each species and age group. Sample sizes are given in Table 2.

Results

In all species and age groups, the feathers were enriched in ^{15}N and ^{13}C compared with blood (Table 2, Fig. 1). The differences ranged between 0.4 and 1.5‰ for $\delta^{15}N$ and between 0.9 and 1.6‰ for $\delta^{13}C$ (Table 2, Fig. 1). In all species except Trindade petrels, the $\delta^{15}N$ and $\delta^{13}C$ values of feathers and blood samples were highly correlated in individuals (Table 2).

Discussion

Our study shows differences between the carbon and nitrogen stable isotopes of blood and simultaneously growing feathers of five species of Procellariiformes, representing 5 genera. We found that feathers were consistently enriched in ^{15}N and ^{13}C compared with blood in all species.

This is in line with previous studies that reported differences in isotope values between blood and feathers (e.g. Hobson and Clark 1992b, Bearhop *et al.* 2000a, Cherel *et al.* 2005a, b). However, the present approach is novel in that it used simultaneously collected samples from wild birds in their natural habitat, and exhibiting their natural foraging behaviours. In contrast, previous studies did not sample the same individuals simultaneously (e.g. Cherel *et al.* 2005a, Bearhop *et al.* 2000a, for simultaneously sampled hydrogen see Langin *et al.* 2007). Further, studies with simultaneous sampling, to our knowledge, have been carried out only in captive birds thus far (Hobson and Clark 1992b, Cherel *et al.* 2005b, but see Bugoni *et al.* 2008a).

The most likely explanation for increased ^{15}N and ^{13}C isotope enrichment in feathers compared to blood are metabolic differences. These may be found in the different protein sources (several proteins in blood, keratin in feathers) and differences would also appear if endogenous reserves are used as precursors during feather synthesis (e.g. Cherel *et al.* 2005a). For example, a large difference between ^{15}N enrichment factors of whole blood and feathers may indicate that most amino acids available for keratin synthesis are provided by endogenous reserves. Conversely, almost identical and low ^{15}N enrichment factors in whole blood and feathers may suggest a dietary origin of feather amino acids (Cherel *et al.* 2005a).

The presence of uric acid, which is ^{15}N depleted (Petersen and Fry 1987), has also been suggested as a cause of isotopic differences between blood and feathers. Bearhop *et al.* (2000b) found that uric acid removal in Great skua *Stercorarius skua* blood increased the measured $\delta^{15}N$ by 1.2‰, while $\delta^{13}C$ was not affected. As Cherel *et al.* (2005a) pointed out, the effect of uric acid is likely to be minimal because of the small amount of uric acid in blood, compared to protein (Garcia-Rodriguez *et al.* 1987, Boismenu *et al.* 1992). Higher values might be found, however, in times of high protein turnover such as during growth in chicks (Bearhop et al 2000b and references therein). In the present study, both the nitrogen and carbon isotope ratios were lower in blood than in feathers, indicating that uric acid is not the only source for the observed difference between tissues. In Thin-billed prions, blood cells rather that whole blood were analysed, and as uric acid is present in the plasma, it should not interfere with the measurements in this case. In fact, we found that the difference between $\delta^{15}N$ of blood and feathers was smallest in Thin-billed prions (0.4‰), and the difference was statistically significant ($P = 0.021$) rather that highly significant ($P < 0.001$) as in all other species. Thus, when the separation of plasma is possible, this may be a good way

to exclude uric acid as an error source and allow better comparison between blood and feathers from the same species.

In four of five species in this study, $\delta^{15}N$ and $\delta^{13}C$ values of feathers and blood samples from individual birds were highly correlated. There was one exception of this pattern. In Trindade petrels we observed a correlation only for adults and only for nitrogen. It is tempting to suggest that this may have resulted from a large variation in foraging areas over a short time, as the Trindade petrel is the only tropical species in our dataset. In tropical species, foraging occurs over vast oceanic areas, and thus there may be more short-term variation in diet, as well as individual metabolic differences (e.g. in the use of internal stores during feather growth). Foraging trips by Trindade petrel parents vary from hours to weeks (Luigi *et al.* 2008), which could suggest that chicks use both diet and endogenous stores for tissue formation, depending on feeding frequencies. Thus the lack of intertissue correlations of carbon and nitrogen isotope values could reveal individual differences according to nutritional status.

In the present study, the difference between blood and simultaneously grown feathers was remarkably similar across five species of Procellariiformes representing five genera (Fig. 1), despite differences in ecology and geographical regions. It was also in the range of the difference found for captive King *Aptenodytes patagonicus* and Rockhopper *Eudyptes chrysocome* penguins, where Cherel *et al.* (2005b) found difference of 1.5 and 1.7‰ for $\delta^{15}N$ and between 0.9 and 1.1 ‰ for $\delta^{13}C$.

In deciding which tissue type is more reliable, both tissue types have error sources. In blood, variable content of uric acid and lipids is of concern, but this may be avoided if red blood cells can be used rather than whole blood, which may also be beneficial in many studies because red blood cells sample a much longer time window than plasma (e.g. Podlesak *et al.* 2005). In feathers, the variable use of endogenous reserves during feather growth has to be taken into account, especially of certain amino acids such as sulphur-containing amino acids (cysteine and methionine) that are abundant in keratin but may be scarce in recently ingested food, such that feather stable isotopes may also be biased. For chicks the role of stored reserves is not usually a major concern, although this needs to be confirmed in species undergoing mass recession during the growth of the feathers, as is the case in Procellariiformes. The best solution may be the analysis of both blood and growing feather so that the two together give a better indication than either tissue alone.

We suggest that future studies consider the following: (i) sample both tissues if possible to overcome limitations of each (ii) use similar tissues to compare temporal patterns when possible - e.g. compare moult feathers with regrowths in the breeding season (iii) RBC may be preferable to whole blood or plasma, by avoiding uric acid effects on nitrogen isotope ratios (iv) correlation between isotopic values of different tissues of similar age should be addressed, which could provide arithmetic corrections and make comparisons across tissues viable (v) more research is needed on metabolic differences

between chick and adults, as well as studies addressing differences in chick and adult diets in the nesting period; (vi) avoid to preserve tissues with preservatives containing the stable isotopes to be analysed, such as ethanol or heparine for determination of SI of carbon in blood.

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Table 1. Species, abbreviations used in Fig. 1 and sampling locations of the Procellariiformes included in the present study.

Species	Abbreviation	Age and Sample location
Atlantic Yellow-nosed Albatross *Thalassarche chlororhynchos*	AYA	Adults and immatures (at sea SW Atlantic)
Spectacled Petrel *Procellaria conspicillata*	SPP	Adults and immatures (at sea SW Atlantic)
Great Shearwater *Puffinus gravis*	GRS	Adults and immatures (at sea SW Atlantic)
Trindade Petrel *Pterodroma arminjoniana*	TRP	Breeding adults (colonies Trindade Island)
Trindade Petrel *Pterodroma arminjoniana*	TRP	Chicks (colonies Trindade Island)
Thin-billed Prion *Pachyptila belcheri*	TBP	Chicks (colonies New Island, Falkland Islands)

Table 2a. Carbon stable isotopes (means ± S.E.) of seabird blood and growing feathers, representing similar time periods, and individual differences between the simultaneously sampled tissues (means ± S.E.)

Species	Age	$\delta^{13}C$				
		Blood	Feathers	Paired differences	Pairwise test	Correlation
Thalassarche chlororhynchos	Adults (N = 15)	-16.8 ± 0.1	-15.5 ± 0.2	1.3 ± 0.1	t = 17.1, P < 0.001	R = 0.894, P < 0.001
Procellaria conspicillata	Adults (N = 21)	-17.1 ± 0.1	-16.0 ± 0.1	1.1 ± 0.1	t = 13.2, P < 0.001	R = 0.807, P < 0.001
Puffinus gravis	Adults (N = 15)	-16.9 ± 0.1	-15.4 ± 0.1	1.4 ± 0.1	t = 18.0, P < 0.001	R = 0.749, P = 0.001
Pterodroma arminjoniana	Adults (N = 18)	-17.5 ± 0.1	-15.9 ± 0.2	1.5 ± 0.2	t = 7.7, P < 0.001	R = 0.036, P = 0.888
Pterodroma arminjoniana	Chicks (N = 15)	-18.3 ± 0.1	-17.2 ± 0.1	1.2 ± 0.2	t = 7.1, P < 0.001	R = 0.080, P = 0.776
Pachyptila belcheri	Chicks (N = 17)	-19.9 ± 0.2	-19.0 ± 0.2	0.9 ± 0.1	t = 13.9, P < 0.001	R = 0.912, P < 0.001

Table 2b. Nitrogen stable isotopes (means ± S.E.) of seabird blood and growing feathers, representing similar time periods, and individual differences between the simultaneously sampled tissues (means ± S.E.)

Species	Age	$\delta^{15}N$				
		Blood	Feathers	Paired differences	Pairwise test	Correlation
Thalassarche chlororhynchos	Adults (N = 15)	16.0 ± 0.4	17.1 ± 0.4	1.1 ± 0.2	t = 6.2, P < 0.001	R = 0.765, P < 0.001
Procellaria conspicillata	Adults (N = 21)	14.3 ± 0.2	15.5 ± 0.2	1.2 ± 0.1	t = 9.7, P < 0.001	R = 0.819, P < 0.001
Puffinus gravis	Adults (N = 15)	14.8 ± 0.3	16.3 ± 0.3	1.5 ± 0.2	t = 6.2, P < 0.001	R = 0.765, P < 0.001
Pterodroma arminjoniana	Adults (N = 18)	11.3 ± 0.2	12.1 ± 0.2	0.8 ± 0.1	t = 5.2, P < 0.001	R = 0.709, P < 0.001
Pterodroma arminjoniana	Chicks (N = 15)	11.3 ± 0.1	12.0 ± 0.2	0.7 ± 0.2	t = 4.0, P = 0.001	R = 0.278, P = 0.316
Pachyptila belcheri	Chicks (N = 17)	12.1 ± 0.2	12.5 ± 0.2	0.3 ± 0.1	t = 2.5, P = 0.021	R = 0.859, P < 0.001

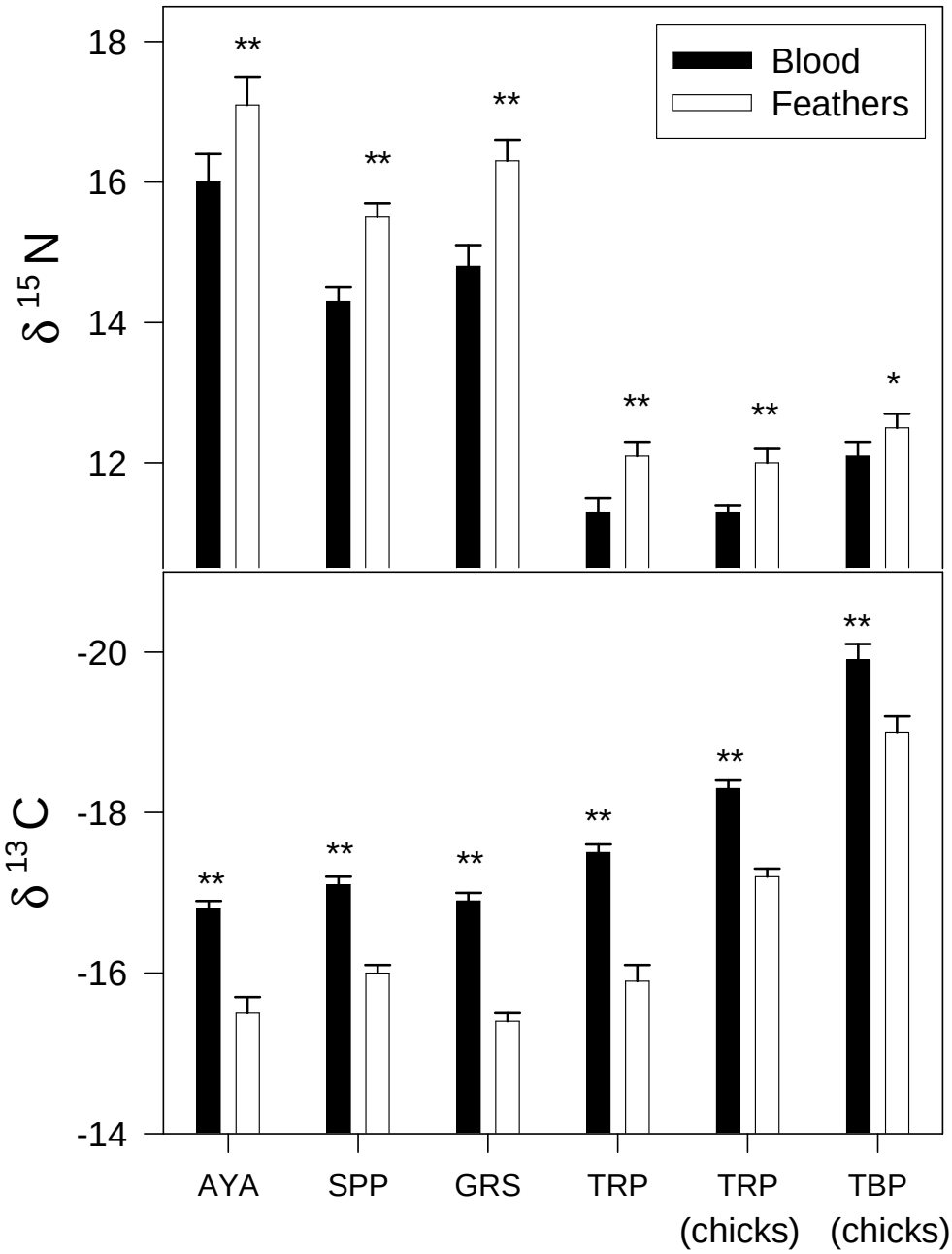

Figure 1. Carbon and nitrogen stable isotopes (means±S.E.) of seabird blood and growing feathers, representing similar time periods. For sample sizes see Table 2.

Appendix 2

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Effects of the First Southern Atlantic Hurricane on Atlantic Petrels (*Pterodroma incerta*)

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ABSTRACT.--We report a massive inland displacement of petrels, particularly female Atlantic Petrels (*Pterodroma incerta*) in southern Brazil, after Hurricane Catarina, the first ever reported hurricane in the South Atlantic Ocean. At least 354 petrels were affected and were found in 26 different locations, up to 420 km from the coast and 1,100 m above sea level. Birds were in heavy molt and near starvation, which probably contributed to their displacement and mortality. *Received 2 October 2006. Accepted 4 March 2007.*

The Atlantic Petrel (*Pterodroma incerta*) is a medium-sized gadfly petrel endemic to Gough and Tristan da Cunha islands (Brooke 2004); it has vulnerable global status (Birdlife International 2004) and is one of the least known seabirds (Cuthbert 2004). The global population is ~1.8 million pairs and declining because of low breeding success (20%) due to predation by introduced house mice (*Mus musculus*) on Gough Island (Cuthbert 2004, Cuthbert and Hilton 2004). It is also predated by Southern Skuas (*Stercorarius antarcticus*) on Gough Island and by rats (*Rattus* spp.) on Tristan da Cunha Island (Birdlife International 2004). The pelagic distribution is largely confined to the South Atlantic Ocean with a few records in the Indian Ocean (Enticott 1991, Brooke 2004). Distribution records range from 01° 31' S, 38° 46' W off Brazil (Bourne and Curtis 1985) to 65° 12' S, 41° 05' W in the Weddell Sea (Orgeira 2001) with most between 20 and 50° S (Enticott 1991). The species is most abundant close to the Subtropical Convergence Zone (Rumboll and Jehl 1977, Veit 1995).

Hurricanes, also called cyclonic storms, typhoons, or cyclones can have severe impacts on populations of vertebrates and invertebrates reducing abundance or extinguishing small populations, as well as extirpating them in more exposed areas (Spiller et al. 1998). Effects on terrestrial birds could be direct, such as death when exposed to strong winds and rain, and displacement to offshore waters, or indirect, by increasing predation rates, destruction of nesting and roosting areas, and reduction of food resources (Wauer and Wunderle 1992, Wiley and Wunderle 1993, Collazo et al. 2003, White et al. 2005). The main effects of hurricanes on seabirds are loss of eggs and mortality of chicks and adults with reduction of breeding success of terns (*Sterna* spp.) and nodies (*Anous* spp.) (White et al. 1976, Langham 1984), direct mortality of adults caused by strong winds (Cely 1991), or petrels and shearwaters displaced inland, particularly in North America (Murphy 1936, Heintzelman 1961, Wiley and Wunderle 1993). Birds found inland are apparently entrapped in the eye of hurricanes and are held away from the periphery of gales (Murphy 1936).

We describe the inland displacement of seabirds after Hurricane Catarina, which hit southern Brazil in March 2004 and provide data on biometry, molt, and body condition of affected Atlantic Petrels. Hurricane Catarina was named after Santa Catarina State in southern Brazil (Fig. 1) and was the first ever reported hurricane in the South Atlantic Ocean (Pezza and Simmonds 2005). It began as an extra-tropical cyclone 800 km from the coast of Brazil (26°S) ~ 20 March 2004 with minimal pressure inside the eye of 974 hPa (hectoPascal) and a total diameter of 400 km (Pezza and Simmonds 2005). It reached the coast of Rio Grande do Sul and Santa Catarina states on 28 March 2004 with winds from 119 to 153 km/hr and was classified as Category I according to the Saffir-Simpson scale (Pezza and Simmonds 2005). According to local newspapers, the winds destroyed 33,000 houses on the Brazilian coast and sank two fishing vessels in offshore waters.

METHODS

Data where birds were found were obtained by contacting rehabilitation centers. Standard measurements (Proctor and Lynch 1993) and molt status of 29 Atlantic Petrels were recorded, and body condition was assessed by necropsies. Measurements are provided as mean ± standard deviation, range, and sample size (n). Wing and tail measurements were not taken from birds molting the outer primaries or central tail feathers, respectively.

OBSERVATIONS

The Atlantic Petrel was the main species affected by Hurricane Catarina. One hundred and twenty-nine Atlantic Petrels and one Spectacled Petrel (*Procellaria conspicillata*) were found inland in Rio Grande do Sul State in an area ~300 km wide and up to 420 km from the coast. They were distributed from sea level to 1,100 m in 26

municipalities of Rio Grande do Sul State (Fig. 1). A flock of 50 Atlantic Petrels also was recovered in a freshwater reservoir used for hydroelectric power production, 190 km from the coast. In addition, another 225 petrels were reported inland in nearby Santa Catarina State, including Giant Petrels (*Macronectes* sp.) and Prions (*Pachyptila* sp.) for which specific identification were not obtained. Birds from Rio Grande do Sul State were cared for at rehabilitation centers, but all Atlantic Petrels died within 2 weeks; only the Spectacled Petrel was released back to sea after 10 days. A minimum of 354 birds was recorded, but probably many more were displaced inland undetected.

Measurements of males and females were pooled (Table 1) because they overlap considerably (Cuthbert 2004). Necropsy of birds revealed the Atlantic Petrels were severely emaciated with mean body mass of 344.6 ± 25.2 g (Table 1) and no fat storage. No abnormality of internal organs was macroscopically detected and parasite infections were recorded in the digestive tract of only two birds from 23 examined.

The sex ratio was strongly biased to females (6 males, 23 females; $\chi^2_{Yates} = 8.83$, $P = 0.003$, df = 1). Females had enlarged ovaries (1-2 mm in length) and 28 of 29 birds were in heavy molt. Twenty-three of 29 birds were molting P9 and/or P10, 26 were molting rectrices (1 to 6 pairs molting simultaneously), and 28 birds (96.6%) had contour body molt. Birds were molting a larger number of rectrix feathers than primary feathers and the pattern of primary molt was more symmetrical than tail molt.

DISCUSSION

To our knowledge, this is the largest number of pelagic seabirds killed by a hurricane, comparable only to the death of 200-400 Brown Pelicans (*Pelecanus occidentalis*) following Hurricane Hugo in 1989 (Cely 1991). This event is not rare in the North Atlantic Ocean where both hurricanes and seabirds in inland areas are more common. Some examples are a Black-capped Petrel (*Pterodroma hasitata*) captured alive in August 1893 in Virginia, USA 2 days after a cyclone. It was found in a fish pond 320 km from the coast and 700 m above sea level, in final molt stage and starving (Smyth 1893). Murphy (1936) described records of Trindade Petrel (*P. arminjoniana*), displaced to Ottawa, Ontario and Ithaca, New York. Heintzelman (1961) recorded a Kermadec Petrel (*P. neglecta*) at Hawk Mountain Lookout, Pennsylvania, USA after Hurricane Gracie in October 1959. In addition, at least three emaciated Atlantic Petrels were collected from a hydroelectric power dam in the eastern Brazilian Amazonia in September 1984 (03° 50’ S, 49° 45’ W), 400 km from the nearest open sea and over 2,000 km from their normal area of occurrence in the western Atlantic Ocean (Teixeira et al. 1986). Overall, *Pterodroma* petrels appear to be more prone to be affected by hurricanes in comparison with other pelagic seabirds.

Measurements were in the range of 54 birds for which data were available from Gough Island (Swales 1965) with the exception of the tarsus, which was consistently longer than for birds from Gough Island (39.1 ± 1.6, 35-43 mm). However, tarsus length

in Brazil agrees with 13 males and females measured by Murphy and Pennoyer (1952), which were in the range of 42.1-45 mm. All measurements were in the range for breeding birds provided by Cuthbert (2004). Differences in tarsus length provided by Swales (1965) in comparison with those reported by Murphy and Pennoyer (1952), Cuthbert (2004), and the present study were probably due to different measurement methods.

The mean body mass of birds affected by Hurricane Catarina of 344.6 g (310 – 410 g) was well below the 522.0 g (440-595 g) reported by Swales (1965) and the 544.4 g (420-720 g) reported by Cuthbert (2004) for birds on Gough Island. Thus, the birds appear to have been starving and could have been suffering from effects of the hurricane for several days. Atlantic Petrels feed largely on squid (87% by mass) (Klages and Cooper 1997) and those examined had obviously not been feeding for several days when inland.

The larger number of females in the sample could be due to differences in at-sea distribution or differences in body condition making females more vulnerable to the storm. The Atlantic Petrel is a winter breeding species arriving in colonies in late March and departing after 3-4 weeks for a pre-laying exodus of ~ 50 days (Cuthbert 2004). Most of the Atlantic Petrels were in heavy molt and petrels in general do not molt during their first year. This information, along with the enlarged size of gonads reported here, suggests that petrels found inland in Brazil were ready to return to colonies when they were entrapped by the hurricane. Early accounts of Tristan da Cunha islanders of birds arriving in colonies still molting (Elliot 1957) agree with our data and suggest that molt is completed during the exodus period and before egg laying, which occurs from 15 June to 21 July (Cuthbert 2004).

Patterns of primary molt were more symmetrical than tail molt, consistent with their vital importance for flight in comparison with tail feathers. Langston and Rohwer (1996) suggested that worn feathers or heavy molt could be dangerous, particularly during storms, and Kinsky (1968) suggested that a stranding of 40 Shy Albatrosses (*Thalassarche cauta*) in 1947 was due to heavy molt. Primary molt in petrels is commonly descendant (Marchant and Higgins 1990) and rectrix molt starts after primary molt (Ginn and Melville 1983). Atlantic Petrels displaced by Hurricane Catarina were in late molt stage, which probably was important in depletion of fat reserves and starvation.

Effects of hurricanes on seabird populations are poorly understood, but could be potentially severe when affecting large numbers of endangered species. Hurricanes in the South Atlantic Ocean are postulated to increase in frequency with global warming (Pezza and Simmonds 2005) with potential detrimental effects on endangered species.

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TABLE 1. Measurements (mm) and body mass (g) of Atlantic Petrels (*Pterodroma incerta*) displaced inland after Hurricane Catarina in southern Brazil in March 2004. Measurements of males (*n* = 6) and females (*n* = 23) were pooled.

	Mean ± SD	**Range**	***n***
Total length	416.6 ± 6.8	404 - 423	9
Wingspan	1,100 ± 20.3	1,080 - 1,130	5
Wing	323.1 ± 7.7	310 - 332	7
Tail	134 ± 11	122 - 154	11
Culmen length	38.1 ± 1.9	35.8 - 41.3	29
Tarsus length	45.4 ± 1.8	42.4 - 51.3	29
Body mass	344.6 ± 25.2	310 - 410	23

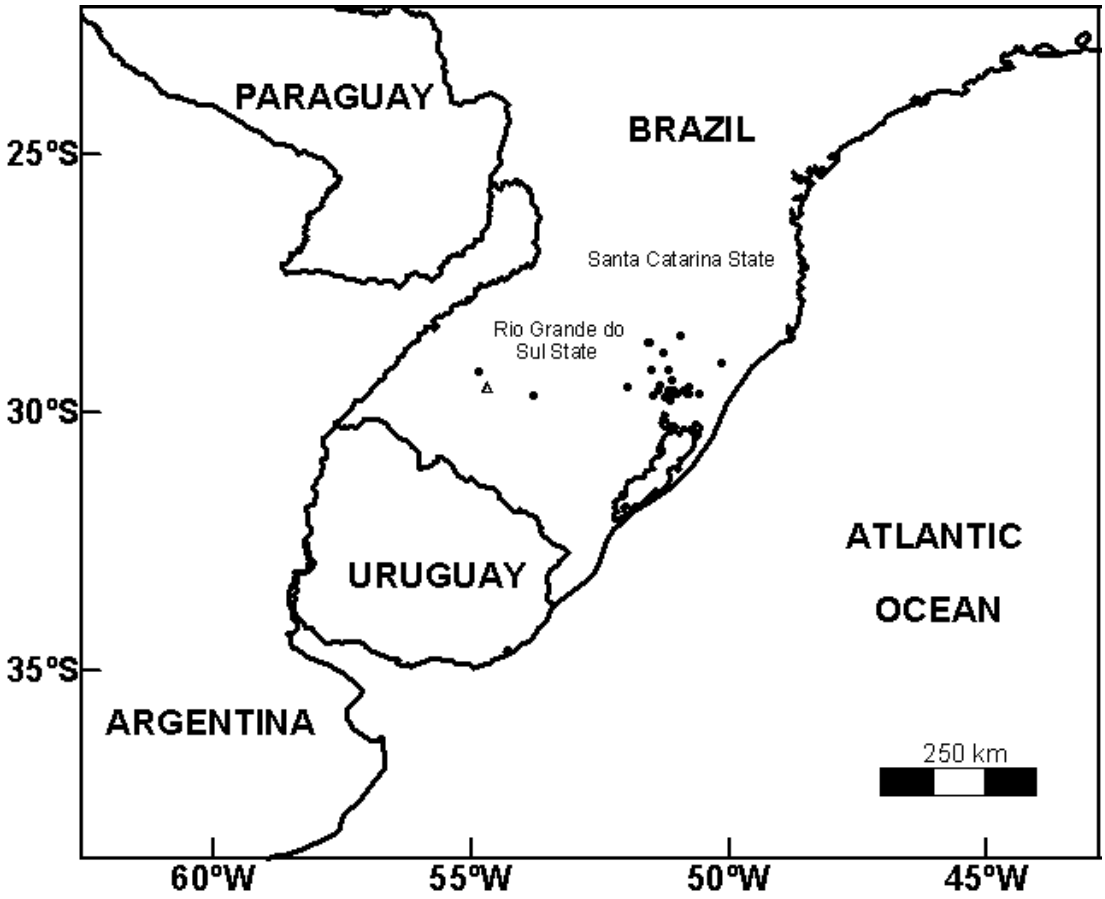

FIG. 1. Inland localities where Atlantic Petrels (dots) and Spectacled Petrel (triangle) were found in Rio Grande do Sul State, southern Brazil after Hurricane Catarina in March 2004.

Appendix 3

REMARKS ON OTHER DATA GATHERED BUT NOT INCLUDED IN THE THESIS

An additional topic intended to be part of the thesis was an analysis of birds attending pelagic longline vessels for discards during hauling. Despite simple information on bird abundance during setting being provided in Chapter 9, and such data being available for the bottom longline in Brazil (Olmos 1997; Olmos & Bugoni 2006) it is not available for the pelagic longline. Based on census and environmental variables collected by onboard observers from 2001 to 2007 during 689 hauls, I aimed to describe how numbers vary during hauling when discard is being released, changes according to discard levels, abiotic variables accounting for seabird numbers (month, SST, chlorophyll, water depth, barometric pressure, and areas). It will fit nicely in the context of the thesis in describing the composition of the community, but due to time constraints it was not included here and remains for future study.

In addition to wintering birds I also investigated the Trindade Petrel (*Pterodroma arminjoniana*) breeding on Trindade Island. During two expeditions and five months of field work I collected a range of information and samples. Again, due to time limitations I could not fully address this subject for the thesis in spite of the considerable effort, time and funds dispensed. I describe here the general aims of this project, preliminary results and difficulties we faced during their development.

When the whole project was designed I based it on a paper of Imber (2004) on specimens from museums, feather lice and calls from which he suggested that the Kermadec Petrel (*Pterodroma neglecta*) also occurred on Trindade Island. Thus I planned a taxonomical and ecological study to compare both species. After long field work (5 months on Trindade Island) and detailed analysis of evidences provided by Imber (2004) I failed to find the Kermadec Petrel on Trindade Island, and doubt that it occurs there (or if in fact it occurred in the past). I planned to elucidate the taxonomy of petrels from Trindade Island, which is a priority for both scientific and conservation reasons as the taxa have a disjunct distribution in Atlantic and Indian Oceans in two single islands and with very small population sizes (Luigi *et al.* 2008). For this I sampled 77 birds (59 adults, 18 chicks), planned to be the base for genetic, biometric, plumage, feather lice, and vocalization study. Because a similar study was simultaneously being carried out by Ruth Brown (Institute of Zoology, London) as part of her PhD, based on samples from Round Island (Indian Ocean), samples from Trindade Islands were provided to her. She focused on genetics, developed primers and ran molecular analysis from both islands in a cooperative project on taxonomy based on genetic, biometry, feather lice, and plumage characteristics. Preliminary results based on 1000bp of the cytochrome-b gene and a neighbour-joining tree using all haplotypes (Round and Trindade Islands) (R. Brown, *in litt.*) show that all haplotypes from Kermadec Petrels (from Round Island) fall into a different clade (along with some Trindade Petrels from Round Island), which could be due

to frequent hybridization occurring on that colony. Round Island haplotypes on their own produce a tree with three reasonably distinguishable clades and when the Trindade Island haplotypes are included they all fall within one of these clades. The third clade contains only one haplotype - found in Round Island *P. arminjoniana* only. However, Trindade Island haplotypes are not exactly the same as any of the Round Island haplotypes (R. Brown*, in litt.*).

Feather lice from both islands were identified by Dr. Ricardo Palma (New Zealand Museum), with Trindade and Kermadec Petrels from Round Island hosting *Halipeurus heraldicus* and Trindade Petrels from Trindade Island hosting *P. kermadecensis*, previously supposed to be an exclusive louse of Kermadec Petrels. The occurrence of *H. kermadecensis* on Trindade birds was previously used to suggest that the Kermadec Petrel breed there (Imber 2004), for which I found no evidence after 5-months of fieldwork. The pattern is complex, but further studies based on feather lice identity and genetics, and morphometric, plumage and vocalizations of birds will be carried out.

I also addressed ecological aspects of petrels from Trindade Island. Basically I collected data on feeding ecology and foraging grounds of the Trindade Petrel based on stable isotopes in blood and feathers, spontaneous regurgitates and geolocators. Geolocation is a relatively new technology used to track animals through large distances. It has some advantages in comparison with satellite transmitters - low costs, small size, larger battery lifespan; and some disadvantages - limited accuracy and the need to recapture the animal to recover transmitters and download data. It has been successfully used to track seabirds during breeding and wintering periods. Preliminary analysis of stable isotopes were carried and included in Appendix 1. All prey from regurgitates were already identified (mainly squids and fish) and data from eight geolocators were used to track breeding birds during foraging trips at sea. Initially, 25 geolocators were deployed, but the unpredictable feeding frequencies and short period of tagged birds spent on the nest (1 to 15 days), nest failures caused by egg and chick predation by the terrestrial crab *Gecarcinus lagostoma*, as well as logistic limitations precluded the recovery of most geolocators. Those geolocators recovered show that the species forages in a vast area of tropical oceans, mainly to the south, reaching ~35°S, feeding frequently at night, which agrees with data on bioluminescent squids being caught. Some general information on mapping colonies, censuses of breeding birds and other general information collected during this study will be available in Luigi *et al.* (2008).